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(54) Title: THE USE OF DOMAINS OF TYPE IV COLLAGEN T INHIBIT ANGIOGENESIS AND TUMOUR GROWTH

(57) Abstract: The instant invention provides methods and kits for inhibiting angiogenesis, tumor growth and metastasis, and endothelial cell interactions with the extracellular matrix, involving contacting the tumor, animal tissue, or endothelial cells with an amount effective to inhibit angiogenesis, tumor growth and metastasis, or endothelial cell interactions with the extracellular matrix of an antagonist of specific integrin receptors.

THE USE OF DOMAINS OF TYPE IV COLLAGEN T INHIBIT ANGIOGENESIS AN TUMOUR GROWTH

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Cross Reference

This application claims priority to U.S. Provisional Application Serial No. 60/127,391 filed April 1, 1999, and is a continuation in part of U.S. Application Serial No. 09/277,665, filed March 26, 1999, which is a continuation in part of U.S. 10 Application Serial No. 09/183,548 filed October 30, 1998, which is a continuation of 08/800,965 filed February 18, 1997, now U.S. Patent No. 5,856,184.

Field of the Invention

This invention relates to methods and kits for inhibiting angiogenesis, tumor 15 growth and metastasis, and endothelial cell interactions with the extracellular matrix.

Background of the Invention

Angiogenesis, the process of formation of new blood vessels, plays an important role in physiological processes such as embryonic and postnatal 20 development, as well as in wound repair. Formation of blood vessels can also be induced by pathological processes involving inflammation (e.g., diabetic retinopathy and arthritis) or neoplasia (e.g., cancer) (Folkman, 1985, Perspect. Biol. Med., 29, 10). Neovascularization is regulated by angiogenic growth factors secreted by tumor or normal cells as well as the composition of the extracellular matrix and by the activity of 25 endothelial enzymes (Nicosia and Ottinetti, 1990, Lab. Invest., 63, 115).

During the initial stages of angiogenesis, endothelial cell sprouts appear through gaps in the basement membrane of pre-existing blood vessels (Nicosia and Ottinetti,

1990, *supra*; Schoefl, 1963, Virehous Arch, Pathol. Anat. 337, 97-141; Ausprunk and Folkman, 1977, Microvasc. Res. 14, 53-65; Paku and Paweletz, 1991, Lab. Invest. 63, 334-346). As new vessels form, their basement membrane undergoes complex structural and compositional changes that are believed to affect the angiogenic response 5 (Nicosia, et. al., 1994, Exp. Biology, 164, 197-206). Early planar culture models have shown that basement membrane molecules modulate the attachment, migration, proliferation, and organizational behavior of endothelial cells (Nicosia, et. al., 1994, *supra*). More recent studies with three-dimensional aortic culture models that more closely simulate angiogenic conditions during wound healing *in vivo* suggest that the 10 basement membrane is a dynamic regulator of angiogenesis, and its function varies according to its molecular components (Nicosia, 1994, *supra*).

A common feature of all solid tumor growth is the requirement for a blood supply. Therefore, numerous laboratories have focused on developing anti-angiogenic compounds based on growth factors and their receptors. While this approach has led to 15 some success, the number of growth factors known to play a role in angiogenesis is large. Therefore, the possibility exists that growth factor antagonists may have only limited use in treating cancer since tumors and associated inflammatory cells likely produce a wide variety of factors that can induce angiogenesis.

In this regard, a strategy that targets a common feature of angiogenesis, such as 20 endothelial cell adhesion to the extracellular matrix (ECM), might be expected to have a profound physiological impact on tumor growth in humans. This notion is supported by the fact that RGD-containing antagonists of the $\alpha v\beta 3$ integrin ECM cell adhesion receptor can block angiogenesis. (U.S. Patent No. 5,766,591) Furthermore, the $\alpha v\beta 3$ integrin is expressed most prominently on cytokine -activated endothelial and smooth

muscle cells and has been shown to be required for angiogenesis. (Varner et al., Cell Adhesion and Communication 3:367-374 (1995); Brooks et al., Science 264:569-571 (1994)). Based on these findings, a potentially powerful new approach to anti-angiogenic therapy might be to specifically target critical regulatory domains within 5 distinct ECM components.

The basement membrane (basal lamina) is a sheet-like extracellular matrix (ECM), which is a basic component of all tissues. The basal lamina provides for the compartmentalization of tissues, and acts as a filter for substances traveling between tissue compartments. Typically the basal lamina is found closely associated with an 10 epithelium or endothelium in all tissues of an animal including blood vessels and capillaries. The basal lamina components are secreted by cells and then self assemble to form an intricate extra-cellular network. The formation of biologically active basal lamina is important to the development and differentiation of the associated cells.

Type IV collagen has been shown to be a major structural component of 15 basement membranes. The protomeric form of type IV collagen is formed as a heterotrimer made up from a number of different subunit chains called α_1 (IV) through α_6 (IV). Up to now, six genetically distinct α -chains belonging to two classes with extensive homology have been identified, and their relative abundance has been demonstrated to be tissue specific. The type IV collagen heterotrimer is characterized 20 by three distinct structural domains: the non-collagenous (NC1) domain at the carboxyl terminus; the triple helical collagenous domain in the middle region; and the 7S collagenous domain at the amino terminus. (Martin, et. al., 1988, Adv. Protein Chem. 39:1-50; Gunwar, et. al. 1991, J. Biol. Chem. 266:14088-14094).

The ability to express recombinant α (IV) NC1 domains provides the opportunity to study the effect of specific domains on many biological processes, such as angiogenesis, tumor metastasis, cell binding to basement membranes, and assembly of Type IV collagen molecules.

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Summary of the Invention

The instant invention provides methods and kits for inhibiting angiogenesis, tumor growth and metastasis, and endothelial cell interaction with the extracellular matrix, each method comprising contacting the tumor, animal tissue, or endothelial cells 10 with antagonists of specific integrin receptors.

Brief Description of the Drawings

Figure 1 illustrates the effects of NC1 (Hexamer) and 7S domains of Type IV collagen at a 50 μ g/ml concentration on angiogenesis from mouse thoracic aorta organ cultures.

15 **Figure 2** illustrates the effects of 7S domain of Type IV collagen on angiogenesis from mouse thoracic aorta organ cultures. The domain concentrations employed in this experiment were 0 μ g/ml (control); 0.5 μ g/ml; 5 μ g/ml and 50 μ g/ml.

20 **Figure 3** illustrates the effects of NC1 (Hexamer) domain of Type IV collagen on angiogenesis from mouse thoracic aorta organ cultures. The domain concentrations employed in this experiment were 0 μ g/ml (control); 5 μ g/ml and 5 μ g/ml and 50 μ g/ml.

Figure 4 are photographs of mouse thoracic aorta segments embedded in Matrigel (EHS basement membrane matrix, Collaborative Biomedical Products, Bedford, MA) at 5 days of culture. Control specimen (0 μ g/ml of NC1 (Hexamer) and 7S domains)

exhibited growth of microvessels from the cultured tissue into the matrix (Figure 4A). In contrast, angiogenesis was inhibited in specimens cultured with 50 µg/ml of 7S domain (Figure 4B) and NC1 (Hexamer) domain (Figure 4C).

5 **Figure 5** is a graphical representation of data demonstrating the *in vivo* effect of IV injection of recombinant ($\alpha 1$) type IV collagen monomer on angiogenesis using fibrin implants in rats.

Figure 6 is a graphical representation of data demonstrating that the recombinant ($\alpha 1$) and ($\alpha 2$) NC1 monomers inhibit the bFGF-induced increase in angiogenic index *in vivo*.

10 **Figure 7** is a graphical representation of demonstrating the dose response effect of recombinant ($\alpha 2$) NC1 monomer on the bFGF-induced increase in total blood vessel branch points *in vivo*.

15 **Figure 8** is a graphical representation of data demonstrating the dose response effect of recombinant ($\alpha 2$) NC1 monomer on the bFGF-induced increase in angiogenic index *in vivo*.

Figure 9 is a graphical representation of data demonstrating the dose response effect of recombinant ($\alpha 2$) NC1 monomer on the bFGF-induced increase in angiogenic index *in vivo*.

20 **Figure 10** is a graphical representation of data demonstrating the effect of recombinant ($\alpha 1$) and ($\alpha 2$) NC1 monomers on mean CS-1 melanoma tumor weight *in vivo*.

Figure 11 is a graphical representation of data demonstrating the dose response effect of recombinant ($\alpha 2$) NC1 monomer on mean CS-1 melanoma tumor weight *in vivo*.

Figure 12 is a graphical representation of data demonstrating the effect of recombinant ($\alpha 1$), ($\alpha 2$), and ($\alpha 4$) NC1 monomers on mean HT1080 tumor weight *in vivo*.

Figure 13 is a graphical representation of data demonstrating the effect of recombinant ($\alpha 1$), ($\alpha 2$), ($\alpha 3$) and ($\alpha 5$) NC1 monomers on mean HEP-3 tumor weight in vivo.

Figure 14 is a graphical representation of data demonstrating human endothelial cell adhesion to immobilized NC1 α monomers.

- 5 **Figure 15** is a graphical representation of data demonstrating the effect of soluble $\alpha 1$ and $\alpha 2$ NC1 monomers on human endothelial cell adhesion to pepsinized collagen type IV.

Figure 16 is a graphical representation of data demonstrating the effect of isolated recombinant NC1 monomers on human endothelial cell migration in vitro.

- 10 **Figure 17 A-F** provides the sequences of each type IV collagen α chain monomer.

Figure 18 is a graphical representation of data demonstrating the effect of monoclonal antibodies against various integrins on human endothelial cell adhesion to recombinant the ($\alpha 2$) NC1 domain.

- 15 **Figure 19** is a graphical representation of data demonstrating human endothelial cell adhesion to the recombinant ($\alpha 1$) NC1 domain.

Description of the Preferred Embodiments

Within this application, unless otherwise stated, the techniques utilized may be found in any of several well-known references such as: *Molecular Cloning: A Laboratory Manual* (Sambrook, et al., 1989, Cold Spring Harbor Laboratory Press), *Gene Expression Technology* (Methods in Enzymology, Vol. 185, edited by D. Goeddel, 1991. Academic Press, San Diego, CA), "Guide to Protein Purification" in *Methods in Enzymology* (M.P. Deutshcer, ed., (1990) Academic Press, Inc.); *PCR Protocols: A Guide to Methods and Applications* (Innis, et al. 1990. Academic Press,

San Diego, CA), *Culture of Animal Cells: A Manual of Basic Technique, 2nd Ed.* (R.I. Freshney. 1987. Liss, Inc. New York, NY), and *Gene Transfer and Expression Protocols*, pp. 109-128, ed. E.J. Murray, The Humana Press Inc., Clifton, N.J.).

As used herein, the term Type IV collagen domain encompasses the group of
5 molecules including the non-collagenous NC1 domain (Hexamer) and 7S collagenous domains, as well as NC1 α chain monomers.

The invention comprises methods for using Type IV collagen NC1 α -monomers (ie: α 1, α 2, α 3, and α 6), which are defined to include such monomers isolated from any multicellular organism or produced via recombinant protein expression from a gene
10 encoding such a monomer from any multicellular organism, and also to encompass various modifications, additions, and/or deletions to such monomers.

In one aspect, the present invention provides methods and kits for inhibiting angiogenesis in an animal tissue comprising contacting the tumor or animal tissue with an amount effective to inhibit angiogenesis of a polypeptide composition comprising one
15 or more isolated type IV collagen NC1 α chain monomers selected from the group consisting of α 1, α 2, α 3, and α 6 NC1 chain monomers.

In another aspect, the present invention provides methods and kits for inhibiting tumor growth in tissue comprising contacting the tumor or tissue with an amount effective to inhibit tumor growth of a polypeptide composition comprising one or more
20 isolated type IV collagen NC1 α chain monomers selected from the group consisting of α 1, α 2, α 3, and α 6 NC1 chain monomers.

In another aspect, the present invention provides methods and kits for inhibiting tumor metastasis in tissue comprising contacting the tumor or tissue with an amount effective to inhibit metastasis of a polypeptide composition comprising one or more

isolated type IV collagen NC1 α chain monomers selected from the group consisting of α 1, α 2, α 3, and α 6 NC1 chain monomers.

In a further aspect, the present invention provides methods and kits for inhibiting endothelial cell interactions with the extracellular matrix in tissue comprising 5 contacting the tumor or tissue with an amount effective to inhibit endothelial cell interactions with the extracellular matrix of a polypeptide composition comprising one or more isolated type IV collagen NC1 α chain monomers selected from the group consisting of α 1, α 2, α 3, and α 6 NC1 chain monomers.

The NC1-encoding domain of each of the six α chain cDNAs has been cloned 10 into a vector for recombinant protein expression as previously described (Sado et al., Kidney Intl. 53:664-671 (1998), incorporated by reference herein in its entirety). The vectors are used to stably transfect human kidney 293 cells, which produce the recombinant protein. The DNA and deduced amino acid sequences of the recombinant type IV collagen alpha chain monomers produced as described are shown in Figure 15 17A-F. The first 17 amino acids correspond to a BM40 signal sequence (which is cleaved from the mature protein), to facilitate protein secretion. All the secreted proteins (ie: mature proteins) start with the sequence APLA followed by the affinity tag, DYKDDDDK at the amino terminus. This tag facilitates purification and identification of the material, and does not interfere with biological activity of the 20 recombinant NC1 α chain monomers.

The type IV collagen NC1 α chain monomers can be produced by any method known in the art, including using recombinant DNA technology or biochemical peptide synthesis technology, or by isolating the NC1 domains from animal sources, such as from basement membrane sources such as bovine lens capsule and bovine kidney glomeruli.

(Peczon et al., Exp. Eye Res. 30:155-165 (1980); Langeveld et al., J. Biol. Chem. 263:10481-10488 (1988); Gunwar et al., J. Biol. Chem. 266:14088-14094 (1991))

In practicing the invention, the amount or dosage range of type IV collagen NC1 α chain monomers employed is one that effectively inhibits angiogenesis, tumor growth, tumor metastasis, and/or endothelial cell-extracellular matrix interactions. An 5 inhibiting amount of NC1 α chain monomers that can be employed ranges generally between about 0.01 $\mu\text{g}/\text{kg}$ body weight and about 10 mg/kg body weight, preferably ranging between about 0.05 $\mu\text{g}/\text{kg}$ and about 5 mg/kg body weight.

The NC1 α chain monomers may be administered by any suitable route, 10 including orally, parentally, by inhalation spray, rectally, or topically in dosage unit formulations containing conventional pharmaceutically acceptable carriers, adjuvants, and vehicles. The term parenteral as used herein includes, subcutaneous, intravenous, intraarterial, intramuscular, intrasternal, intratendinous, intraspinal, intracranial, intrathoracic, infusion techniques or intraperitoneally. In preferred embodiments, the 15 NC1 α chain monomers are administered intravenously or subcutaneously.

The NC1 α chain monomers may be made up in a solid form (including granules, powders or suppositories) or in a liquid form (*e.g.*, solutions, suspensions, or emulsions). The NC1 α chain monomers of the invention may be applied in a variety 20 of solutions. Suitable solutions for use in accordance with the invention are sterile, dissolve sufficient amounts of the NC1 α chain monomers, and are not harmful for the proposed application.

The NC1 α chain monomers may be subjected to conventional pharmaceutical operations such as sterilization and/or may contain conventional adjuvants, such as preservatives, stabilizers, wetting agents, emulsifiers, buffers etc.

For administration, the NC1 α chain monomers are ordinarily combined with one or more adjuvants appropriate for the indicated route of administration. The compounds may be admixed with lactose, sucrose, starch powder, cellulose esters of alkanoic acids, stearic acid, talc, magnesium stearate, magnesium oxide, sodium and calcium salts of phosphoric and sulphuric acids, acacia, gelatin, sodium alginate, polyvinylpyrrolidine, and/or polyvinyl alcohol, and tableted or encapsulated for conventional administration. Alternatively, the compounds of this invention may be dissolved in saline, water, polyethylene glycol, propylene glycol, carboxymethyl cellulose colloidal solutions, ethanol, corn oil, peanut oil, cottonseed oil, sesame oil, tragacanth gum, and/or various buffers. Other adjuvants and modes of administration are well known in the pharmaceutical art. The carrier or diluent may include time delay material, such as glyceryl monostearate or glyceryl distearate alone or with a wax, or other materials well known in the art.

The present invention may be better understood with reference to the accompanying examples that are intended for purposes of illustration only and should not be construed to limit the scope of the invention, as defined by the claims appended hereto.

Example 1 – In Vitro Effect on Angiogenesis

With modifications, the procedures of Nicosia and Ottinetti (1990), *supra*, and Nicosia, et. al. (1994), *supra*, were utilized for experiments designed to test the effect of Type IV collagen on angiogenesis under *in vitro* conditions. The model has been used to study the effects of growth factors and extracellular matrix molecules on the

angiogenic response and employs aortic rings cultures in three-dimensional collagen gels under serum-free conditions. These experiments are outlined below.

A. Methods

Experiments were performed with 1-3 month old Swiss Webster male mice.
5 Following anesthesia, the thoracic aorta was excised under aseptic conditions and transferred to sterile MCDB 131 sterile growth medium (Clonetics, San Diego, CA) containing antibiotics. Fat was dissected away from the aorta and approximately six to eight 1 mm thoracic segments were obtained from each specimen. Segments were transferred to 48 well tissue culture plates. The wells of these plates were layered with
10 100 microliters of Matrigel (EHS basement membrane, Collaborative Biomedical Products, Bedford, MA) prior to transfer of the aortic segments. The Matrigel was diluted 1:1 with MCDB 131 growth medium prior to use. The segments were centered in the wells and an additional 100 microliters of Matrigel was then placed over the specimens. The aortic segments were therefore embedded in the basement membrane
15 matrix. Each well then received 300 microliters of MCDB 131 growth medium. The plates were placed in an incubator maintained at 37° C with 5% CO₂. Specimens were observed daily over a 7 day period. Newly growing microvessels were counted using an inverted phase microscope at various times during the culture period, but data is expressed at 3 and 5 days of culture. To test for the effect of Type IV collagen on
20 angiogenesis, domains at known concentrations were mixed with the Matrigel and with the MCDB 131 growth medium. Fresh MCDB 131 growth medium (plus and minus collagen domains) was changed every 3 days.

B. Results

After establishing the time course of angiogenesis under control conditions (Matrigel plus MCDB 131 growth medium), experiments were performed using various concentrations of Type IV collagen (isolated from bovine lens) NC1 (hexamer) and 7S domains. Data represents the analysis of at least 3 specimens per experimental condition. In the first experiment (**Figure 1**), analysis indicated that at a concentration of 50 µg/ml, NC1 domain and 7S domain significantly inhibited angiogenesis as monitored at 3 and 5 days of culture. In the second experiment, various concentrations of these domains were analyzed. As indicated in **Figure 3**, 7S domain at 50 µg/ml again significantly inhibited angiogenesis at 3 and 5 days. Inhibition was reduced at 5 and 0.5 µg/ml concentrations. As indicated in **Figure 2**, NC1 domain was less effective in blocking angiogenesis as compared to that observed in the first experiment (**Figure 1**), although it was still effective. In addition, as compared to the 7S domain, there was less of a correlation between concentration and inhibitory action.

Figure 4A-C are photographs of mouse thoracic aorta segments embedded in Matrigel (EHS basement membrane matrix, Collaborative Biomedical Products, Bedford, MA) at 5 days of culture in the presence or absence of 50 µg/ml of Type IV collagen domains. The control specimen (no domains) exhibited growth of microvessels from the cultured tissue into the matrix (**Figure 4A**). In contrast, angiogenesis inhibition was observed in tissues cultured in the presence of 50 µg/ml of 7S (**Figure 4B**) and NC1 (Hexamer) domain (**Figure 4C**).

Example 2. Subcutaneous fibrin implant angiogenesis

Recombinant human type IV collagen NC1 ($\alpha 3$) monomer (Sado et al., Kidney International 53:664-671 (1998)) was injected intravenously in Fisher 344 rats containing fibrin implants surgically placed subcutaneously, a modified version of the method described by Dvorak et al (Lab. Invest. 57(6):673-686 (1987)). The implants 5 were then removed and directly analyzed using an inverted microscope. The analysis involved counting the number of blood vessels that had grown into the fibrin in the control and experimental group.

Briefly, 4 fibrin implants were surgically implanted subcutaneously into Fisher 344 rats (2 dorsal and 2 ventral sides). The average rat weight was approximately 125 10 grams.

Three rats (EXP) were given tail vein injections of either control (fibrin alone), 100 μ l of 100 μ g/ml of 7S domain of type IV collagen (approximately 0.80 mg/kg body weight), 100 μ l of 100 μ g/ml of type IV collagen hexamer (approximately 0.80 mg/kg body weight), or recombinant collagen type IV NC1 ($\alpha 3$) monomer at a concentration 15 of 1.26 mg/ml in PBS (120 μ g protein, or approximately 0.96 mg/kg body weight) and 3 rats (C) were given 100 μ l tail vein injections of PBS. Injections of recombinant protein were given every other day for five doses. The injection schedule was as follows:

20 Day 1: (implant day) injection and remove blood sample (EXP and C)
Day 3: Injection (EXP and C)
Day 5: Injection and remove blood sample (EXP and C)
Day 7: Injection (EXP and C)
Day 9: Injection and remove blood sample (EXP and C)
Day 11: Remove and fix implants (save blood sample) (EXP and C)

25

The results of one experiment were as follows:

2 week in vivo experiment:

Control (fibrin alone)

about 66 BV

7S domain of type IV lens collagen (100 µg/ml)	None
Hexamer of type IV lens collagen (100 µg/ml)	None
Monomer (α 3)	None

5 The results are shown as the mean number of blood vessels per implant. The results of this study demonstrate that isolated domains of type IV collagen, including the α 3 monomer, can significantly inhibit capillary growth in the in vivo fibrin clot implant model. In subsequent experiments, the inhibitory effect was occasionally seen to attenuate with time, suggesting that higher dosages or more frequent injections might
10 be even more effective.

A similar experiment was conducted using recombinant human type IV collagen NC1 (α 1) monomer (100 µl of a 1 µg/µl solution; approximately 0.80 mg/kg body weight) and comparing the number of blood vessels that had grown into the fibrin at day 11 of treatment relative to the control group. Three rats per group were analyzed
15 with each rat having 4 implants. These experiments demonstrated that administration of the α 1 monomer significantly inhibited capillary growth in the in vivo fibrin clot implant model (Figure 5).

Example 3. Recombinant NC1 (α 2) domain inhibits angiogenesis in vivo

20 We next tested the effects of systemic administration of soluble NC1 α -chain monomers in the chick embryo CAM angiogenesis assay.

Angiogenesis was induced in the CAMs of 10 day old chick embryos with bFGF as described (Brooks et al., Cell 92:391-400 (1998)). Twenty four hours later the
embryos were systemically treated with various concentrations of recombinant NC1 α -
25 chain monomers, in a total volume of 100 µl of sterile phosphate buffered saline (PBS). Two days later the embryos were sacrificed and the filter discs and CAM tissues

removed. Angiogenesis was quantitated by counting the number of angigenic blood vessel branch points in the confined area of the filter disc. The Angiogenic Index is defined as the number of branch points from experimental treatment minus control treatment.

5 In initial experiments, recombinant α 1 or α 2 NC1 domains were injected at a concentration of 50 μ g per embryo. At this concentration, the NC1 domains were shown to be highly toxic as demonstrated by greater than 90% embryo cell death. However, at lower doses they were well tolerated and showed potent anti-angiogenic activity. A total of 6 individual angiogenesis experiments were conducted with the
10 NC1 domains. However, in two experiments, the bFGF induction was low, making it difficult to interpret the results. The NC1 α 2 domain appeared to be more consistent and potent than the α 1 NC1 domain at inhibiting angiogenesis. In fact, systemic administration of 30 μ g of NC1 α 2 consistently inhibited angiogenesis by greater than 90% (**Figures 6-9**), as measured by inhibition of the bFGF-induced increase in the
15 angiogenic index and the mean number of blood vessel branch points. In contrast, NC1 α 1 domain showed variable inhibitory activity (0%-50%) throughout the experiments.

Example 4 . Recombinant NC1 domain inhibits melanoma tumor growth in vivo:

20 Since the growth of all solid tumors depends on angiogenesis to provide nutrients for its continued expansion, reagents that have the capacity to inhibit angiogenesis may significantly inhibit tumor growth. Therefore, we tested the effects of recombinant NC1 domains of type IV collagen for their effects on tumor growth in
25 vivo.

To test the effects of NC1 domains on tumor growth *in vivo*, we utilized the chick embryo tumor growth assay. Briefly, single cell suspensions of 3 distinct tumor types were applied to the CAM of 10 day old chick embryos. The tumors included CS-1 Melanoma cells (5×10^6), HT1080 human fibrosarcoma cells (4×10^5) and Hep-3 human epidermoid carcinoma cells (2×10^5). The embryos were injected systemically with varying concentrations of NC1 α -chain monomers 24 hours later. The embryos were next allowed to incubate for a total of 7 days, at which time they were sacrificed. The resulting tumors were resected and wet weights determined. A total of 6 tumor growth assays were conducted with the 3 distinct tumor types. A single injection of 10 μ g NC1 α 2 domain inhibited CS1 melanoma tumor growth by approximately 70% relative to control (**Figure 10**). In similar experiments, dose response curves were completed with CS-1 tumors. Systemic administration of NC1 α 2 resulted in a dose-dependent inhibition of CS-1 melanoma tumor growth *in vivo* with a maximum inhibition following a single dose at 30 μ g (**Figure 11**). Systemic administration of 15 NC1 α 1 also inhibited CS-1 tumor growth but it was variable and in some experiments failed to inhibit tumor growth (See **Figure 10**). In similar experiments, NC1 α 2 inhibited HT1080 human fibrosarcoma tumor growth by approximately 50% after a single systemic injection of 30 μ g, while NC1 α 1 and α 4 had no effect (**Figure 12**). Finally, systemic administration of NC1 α 2 (30.0 μ g) and α 3 inhibited Hep-3 human 20 epidermoid carcinoma tumor growth by approximately 40% and 60% respectively, and α 1 inhibited Hep-3 tumor growth by approximately 30%, while NC1 α 5 domain failed to inhibit tumor growth (**Figure 13**).

We conclude from these *in vivo* studies that tumor growth can be inhibited by isolated NC1 α -chain monomers. These molecules can thus be used alone, or to

complement the use of existing anti-tumor agents, in providing enhanced and more effective anti-tumor therapy.

Example 5. Immobilized NC1 domains support human endothelial cell adhesion

5 In order for new blood vessels to form, endothelial cells must have the capacity to adhere and migrate through the ECM. Moreover, this endothelial cell-ECM interaction may facilitate signal transduction events required for new blood vessel formation. Therefore, since type IV-collagen is an ECM protein which is known to support cell adhesion, we tested the ability of the NC1 domains to support endothelial 10 cell attachment.

Microtiter plates were coated with 25 µg/ml of purified NC1 domains followed by incubation with 1% bovine serum albumin (BSA) to block non-specific interactions. Human endothelial cells (ECV304) were then allowed to attach to the immobilized NC1 domains for 1 hour. Non-adherent cells were removed by washing and attached 15 cells were quantified by measuring the optical density (O.D.) of crystal violet eluted from attached cells. Data bars represent the mean +/- standard error of the O.D. from triplicate wells.

Immobilized NC1 α 2, α 3, and α 6 domains supported endothelial cell adhesion while NC1 α 1, α 4, and α 5 domains promoted little if any cell adhesion (**Figure 14**). 20 Soluble NC1 α 1 (a1) and α 2 (a2) inhibited endothelial cell adhesion to pepsinized collagen type IV by approximately 50% (**Figure 15**).

Taken together, these findings demonstrate that isolated, recombinant NC1 domains from the α 1, α 2, α 3, and α 6 chains of collagen type IV can mediate human endothelial cell adhesion and/or inhibit endothelial cell adhesion to ECM proteins in

vitro, and suggest that the potent anti-angiogenic and anti-tumor activity of the isolated NC1 domains is due to disruption of endothelial cell interaction with the extracellular matrix that are necessary for angiogenesis.

5 **Example 6. Endothelial Cell Migration**

Invasive cellular processes such as angiogenesis and tumor metastasis also require cellular motility. Thus we evaluated the ability of isolated NC1 domains to support human endothelial cell migration in vitro. These experiments were conducted essentially according to the methods in Brooks et al., J. Clin. Invest. 99:1390-1398
10 (1997).

The results of these experiments indicate that NC1 α 2, α 3, and α 6 domains can support human endothelial cell migration in vitro, while α 1, α 4, and α 5 domains showed little if any capacity to support endothelial cell migration (**FIG 16**).

15 **Example 7. Efficacy in Lewis lung in vivo tumor**

The above studies indicated that specific domains of collagen type IV can promote cell migration in vitro. Thus, we evaluated the ability of NC1 domains to support endothelial cell migration in vivo.

The α (IV) NC1 domain hexamer, isolated by enzymatic digestion of bovine
20 lens capsule basement membrane by known protocols (Peczon et al., Exp. Eye Res. 30:155-165 (1980)) was tested in the metastatic Lewis lung mouse tumor model using a standard protocol which is considered to be a good model of both metastasis and angiogenesis of lung tumors. (See for example, Teicher et al., Anticancer Res.

18:2567-2573 (1998); Guibaud et al., Anticancer Drugs 8:276-282 (1997); Anderson et al., Cancer Res. 56:715-718 (1996)).

Each study consisted of an untreated control group and six treatment groups. There were ten animals per treatment group with 40 mice in the control. In each study, 5 all treatment was administered intravenously once every 2 days for 7 doses starting one day after tumor inoculation. Dosages of α (IV) NC1 hexamer were either 100 μ g/mouse or 200 μ g/mouse. In the Lewis lung study, the tumor cell inoculum was 1 $\times 10^6$ viable cells. All animals were weighed twice a week throughout the study. Starting one day after the last treatment, 5 mice were periodically sacrificed from each 10 control group to measure pulmonary tumor burden. The experiment was terminated at day 14 when the lungs of the control animals had sufficient tumor mass to provide meaningful evaluation. At that time, the lungs of all remaining animals were excised, weighed, and the number of tumor foci greater than 2 mm in diameter counted. The resulting data showed that both dosages of α (IV) NC1 hexamer significantly reduced 15 the number of visible lung metastases (Mann-Whitney Rank Sum Test, $p < 0.05$), with 8 visible lung metastases in the control, vs. 5 (100 μ g/mouse) and 4 (200 μ g/mouse), and the 100 μ g/mouse dosage reduced the lung weights from a median of 520 mg in controls to a median of 462 mg in experimental, while the median lung weight of mice treated with 200 μ g/mouse was 620 mg.

20 Other in vivo studies demonstrated that tumor cell metastasis to the lung can be reduced by 50% or more using intravenous injections of the Type IV collagen domains in murine B16 melanoma, human A375SM melanoma xenografts. Furthermore, injection of the NC1 hexamer also significantly reduced the number of lung tumors in separate Lewis Lung tumor studies.

Example 8. Defining the Integrin Receptor Mediating Cellular Adhesion to the NC1 domains

To define the integrin receptors that mediated cellular adhesion to the NC1 α 1 and α 2 domains, adhesion assays were performed as described in Example 5 in the presence or absence of function blocking monoclonal antibodies directed to specific integrins (**Figures 18 (α 2); Fig. 19 (α 1)**). These antibodies were directed against α 5 β 3 integrin (anti-avb3), the α 5 β 5 integrin (anti-avb5), the β 1 integrin (b1) (all described in U.S. Patent No. 5,766,591, incorporated by reference herein in its entirety), and monoclonal antibodies directed against the α 1 (anti-a1), α 2 (anti-a2), and α 3 (anti-a3) integrins (purchased from Chemicon, California). These studies indicated that human endothelial cells interact with NC1 α 2 domain primarily through α v β 5 and α v β 3 integrins with variable contribution from β 1 integrins (**Figure 18**). In similar experiments, anti- β 1 integrin antibodies showed a lesser effect on endothelial cell adhesion to NC1 α 2, suggesting a lesser contribution of β 1 integrins to this adhesive activity. In contrast, endothelial cell adhesion promoted by NC1 α 1 domain was mediated by integrin α 3 β 1 (**Figure 19**).

Previous studies have demonstrated that RGD-containing antagonists of the α v β 3 receptor can block angiogenesis (U.S. Patent No. 5,766,591), but the instant invention provides the first demonstration of a non-RGD containing antagonist of the α v β 3 integrin that can block angiogenesis. The present study also demonstrates that antagonists of the α v β 5 integrin and the α 3 β 1 integrins can block angiogenesis.

Thus, the instant invention also provides methods and kits for inhibiting angiogenesis, tumor growth and metastasis, and endothelial cell interaction with the extracellular matrix, each method comprising contacting the tumor, animal tissue, or

endothelial cells with antagonists of specific integrin receptors. Specifically, the methods comprise contacting the tumor, animal tissue, or endothelial cells with one or more of the following polypeptide compositions:

- (a) a polypeptide composition comprising one or more non-RGD containing integrin $\alpha v\beta 3$ antagonists; or
- 5 (b) a polypeptide composition comprising one or more antagonists of $\alpha v\beta 5$ integrin; or
- (c) a polypeptide composition comprising one or more antagonists of $\beta 1$ integrins; or
- 10 (d) a polypeptide composition comprising one or more antagonists of $\alpha 3\beta 1$ integrins.

We conclude from all of the above studies that angiogenesis, tumor growth and metastasis, and endothelial cell adhesion to the ECM, can be inhibited by isolated, recombinant domains of type IV collagen, or by antagonists of specific integrin receptors. The present invention is thus broadly applicable to a variety of uses which include inhibition of angiogenesis and treatment of diseases and conditions with accompanying undesired angiogenesis, such as solid and blood-borne tumors including but not limited to melanomas, carcinomas, sarcomas, rhabdomyosarcoma, 15 retinoblastoma., Ewing sarcoma, neuroblastoma, osteosarcoma, and leukemia.

The invention is further applicable to treating non-tumorigenic diseases and conditions with accompanying undesired angiogenesis, including but not limited to diabetic retinopathy, rheumatoid arthritis, retinal neovascularization, choroidal neovascularization, macular degeneration., corneal neovascularization, retinopathy of

prematurity., corneal graft rejection, neovascular glaucoma., retrobulbar fibroplasia, epidemic keratoconjunctivitis, Vitamin A deficiency, contact lens overwear, atopic keratitis, superior limbic keratitis, pterygium keratitis sicca, sogrens, acne rosacea, phylectenulosis, syphilis, Mycobacteria infections, lipid degeneration, chemical burns,
5 bacterial ulcers, fungal ulcers, Herpes simplex infections, Herpes zoster infections, protozoan infections, Kaposi's sarcoma, Mooren ulcer, Terrien's marginal degeneration, marginal keratolysis, traum, systemic lupus, polyarteritis, Wegeners sarcoidosis, scleritis, Steven's Johnson disease, radial keratotomy, sickle cell anemia, sarcoid, pseudoxanthoma elasticum, Pagets disease, vein occlusion, artery occlusion, carotid
10 obstructive disease, chronic uveitis, chronic vitritis, Lyme's disease, Eales disease, Bechets disease, myopia, optic pits, Stargarts disease, pars planitis, chronic retinal detachment, hyperviscosity syndromes, toxoplasmosis, post-laser complications, abnormal proliferation of fibrovascular tissue, hemangiomas, Osler-Weber-Rendu, acquired immune deficiency syndrome, ocular neovascular disease, osteoarthritis,
15 chronic inflammation, Crohn's disease, ulcerative colitis, psoriasis., atherosclerosis, and pemphigoid. See U.S. Patent No. 5,712,291)

The invention is also broadly applicable to methods for inhibiting tumor growth and metastasis, reduction of scar tissue formation, reduction of complications due to cell adhesion in organ transplants, and the inhibition of lymphocyte adhesion and
20 mobility.

While the fundamental novel features of the invention have been shown and described, it will be understood that various omissions, substitutions, and changes in the form and details illustrated may be made by those skilled in the art without departing from the spirit of the invention. For example, various modifications,

additions, and/or substitutions can be made to the type IV collagen α monomer chains that would be encompassed by the invention.

We claim

1. A method for inhibiting angiogenesis in an animal tissue comprising contacting the tumor or animal tissue with an amount effective to inhibit angiogenesis of a polypeptide composition comprising one or more non-RGD containing integrin $\alpha v\beta 3$ antagonists.
5
2. A method for inhibiting angiogenesis in an animal tissue comprising contacting the tumor or animal tissue with an amount effective to inhibit angiogenesis of a polypeptide composition comprising one or more antagonists of $\alpha v\beta 5$ integrin.
3. A method for inhibiting angiogenesis in an animal tissue comprising contacting the tumor or animal tissue with an amount effective to inhibit angiogenesis of a polypeptide composition comprising one or more antagonists of $\beta 1$ integrins.
10
4. A method for inhibiting angiogenesis in an animal tissue comprising contacting the tumor or animal tissue with an amount effective to inhibit angiogenesis of a polypeptide composition comprising one or more antagonists of $\alpha 3\beta 1$ integrin.
5. A method for inhibiting endothelial cell adhesion to extracellular matrix, comprising contacting the endothelial cell with an amount effective to inhibit endothelial cell adhesion to extracellular matrix of a polypeptide composition comprising one or more non-RGD containing integrin $\alpha v\beta 3$ antagonists.
15
6. A method for inhibiting endothelial cell adhesion to extracellular matrix, comprising contacting the endothelial cell with an amount effective to inhibit endothelial cell adhesion to extracellular matrix of a polypeptide composition comprising one or more antagonists of $\alpha v\beta 5$ integrin.
20
7. A method for inhibiting endothelial cell adhesion to extracellular matrix, comprising contacting the endothelial cell with an amount effective to inhibit endothelial

cell adhesion to extracellular matrix of a polypeptide composition comprising one or more antagonists of $\alpha 3\beta 1$ integrin.

8. A method for inhibiting endothelial cell adhesion to extracellular matrix, comprising contacting the endothelial cell with an amount effective to inhibit endothelial cell adhesion to extracellular matrix of a polypeptide composition comprising one or more antagonists of $\beta 1$ integrins.

9. A method for inhibiting tumor metastasis in tissue comprising contacting the tumor or tissue with an amount effective to inhibit tumor metastasis of a polypeptide composition comprising one or more non-RGD containing integrin $\alpha v\beta 3$ antagonists.

10. 10. A method for inhibiting tumor metastasis in tissue comprising contacting the tumor or tissue with an amount effective to inhibit tumor metastasis of a polypeptide composition comprising one or more antagonists of $\alpha v\beta 5$ integrin.

11. 11. A method for inhibiting tumor metastasis in tissue comprising contacting the tumor or tissue with an amount effective to inhibit tumor metastasis of a polypeptide composition comprising one or more antagonists of $\alpha 3\beta 1$ integrin.

12. 12. A method for inhibiting tumor metastasis in tissue comprising contacting the tumor or tissue with an amount effective to inhibit tumor metastasis of a polypeptide composition comprising one or more antagonists of $\beta 1$ integrins.

13. 13. A method for inhibiting tumor growth in tissue comprising contacting the tumor or tissue with an amount effective to inhibit tumor growth of a polypeptide composition comprising one or more non-RGD containing integrin $\alpha v\beta 3$ antagonists.

14. 14. A method for inhibiting tumor growth in tissue comprising contacting the tumor or tissue with an amount effective to inhibit tumor growth of a polypeptide composition comprising one or more antagonists of $\alpha v\beta 5$ integrin.

15. A method for inhibiting tumor growth in tissue comprising contacting the tumor or tissue with an amount effective to inhibit tumor growth of a polypeptide composition comprising one or more antagonists of $\alpha 3\beta 1$ integrin.
16. A method for inhibiting tumor growth in tissue comprising contacting the tumor or tissue with an amount effective to inhibit tumor growth of a polypeptide composition comprising one or more antagonists of $\beta 1$ integrins.
5

1/30

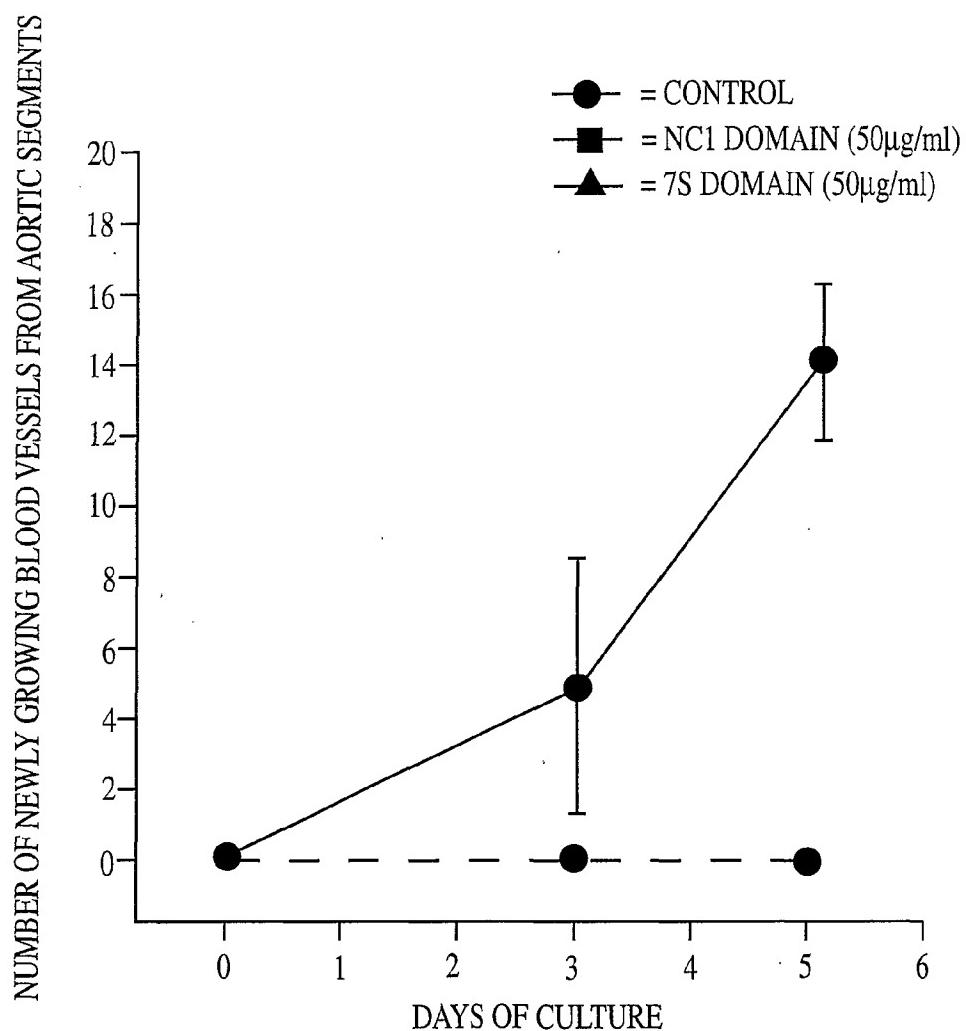


FIG. 1

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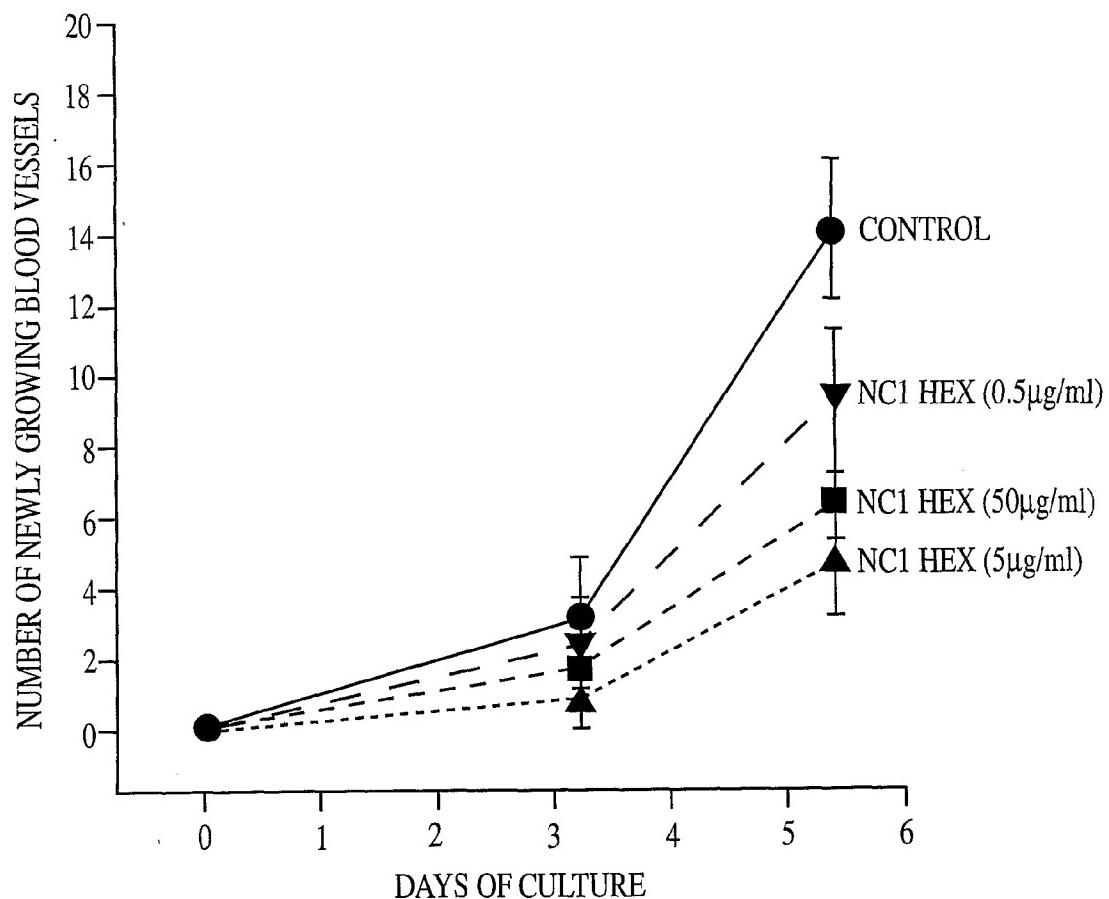


FIG. 2

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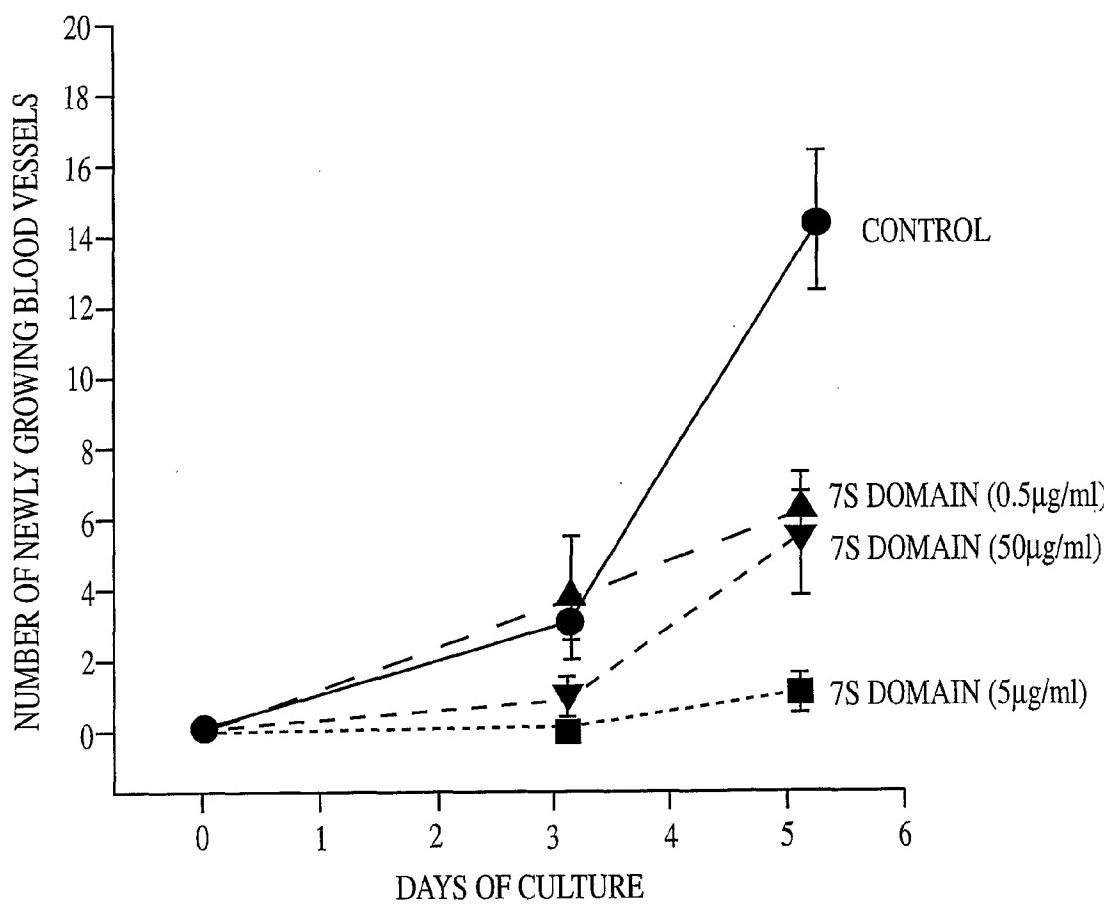
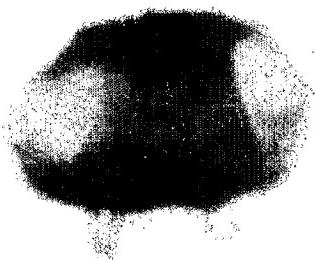
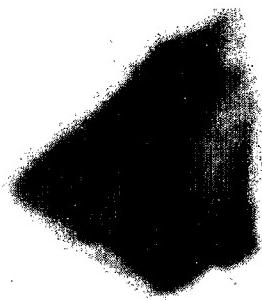


FIG. 3

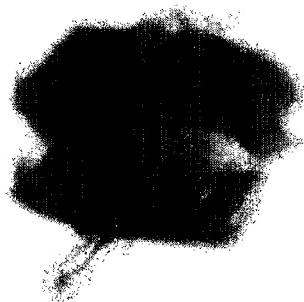
4/30



CONTROL
FIG. 4a



7S DOMAIN (50 $\mu\text{g}/\text{ml}$)
FIG. 4b



NC1 DOMAIN (50 $\mu\text{g}/\text{ml}$)
FIG. 4c

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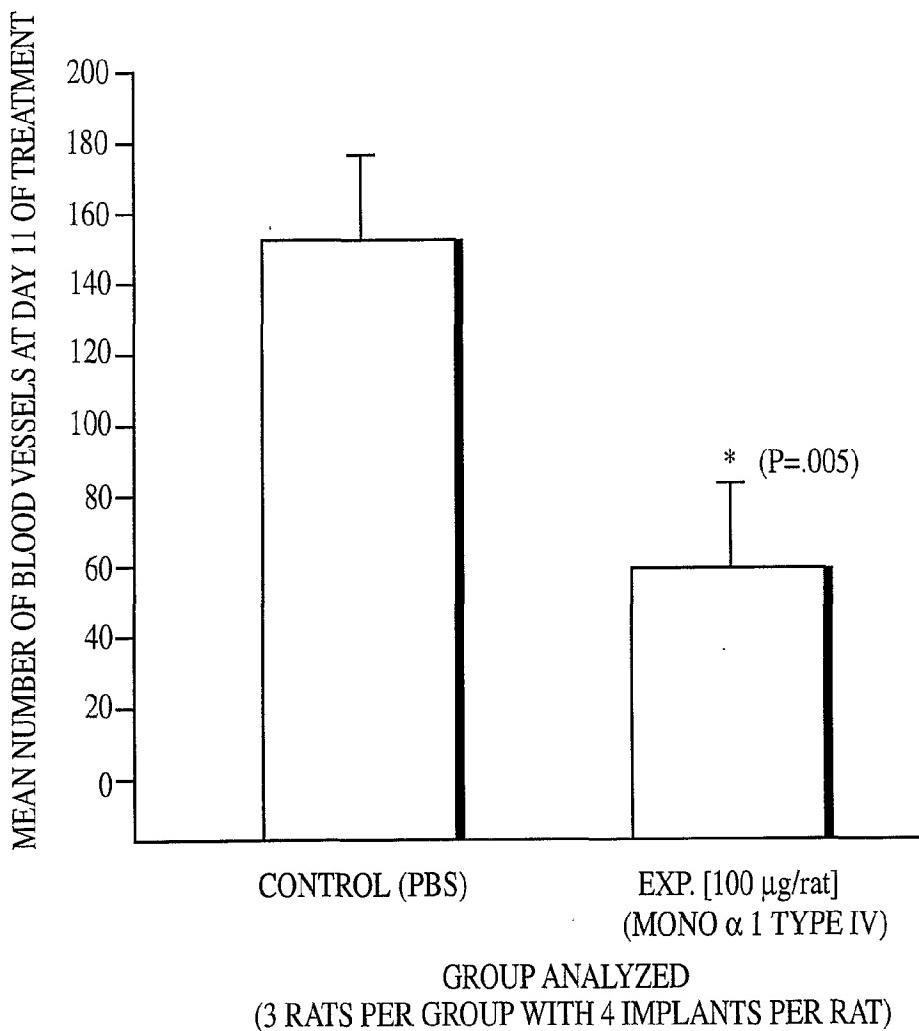


FIG. 5

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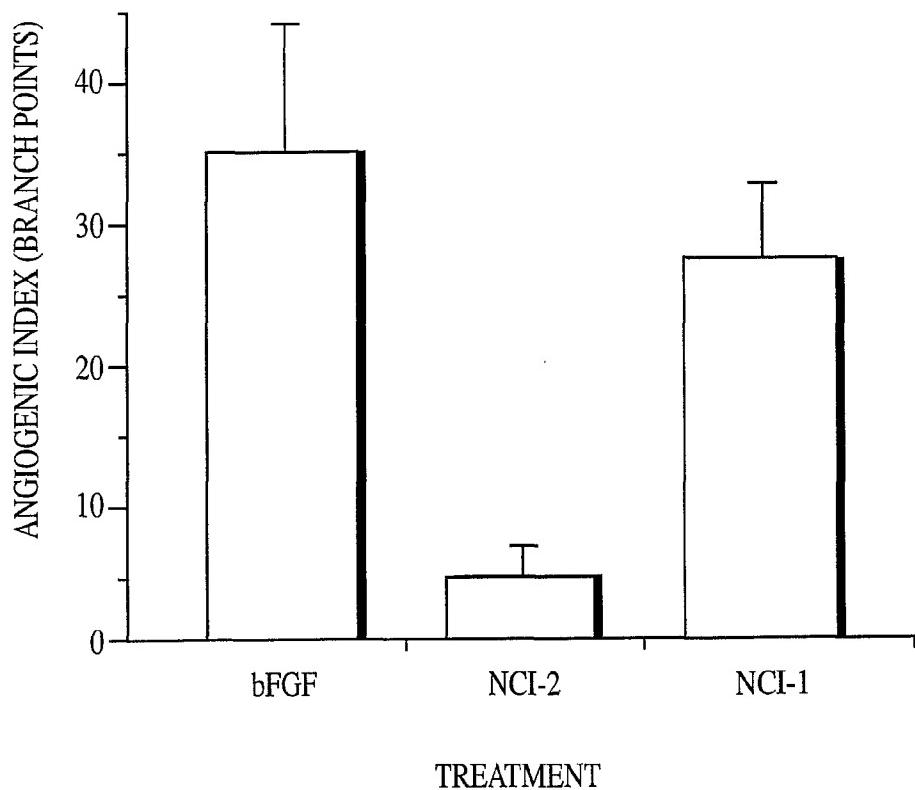


FIG. 6

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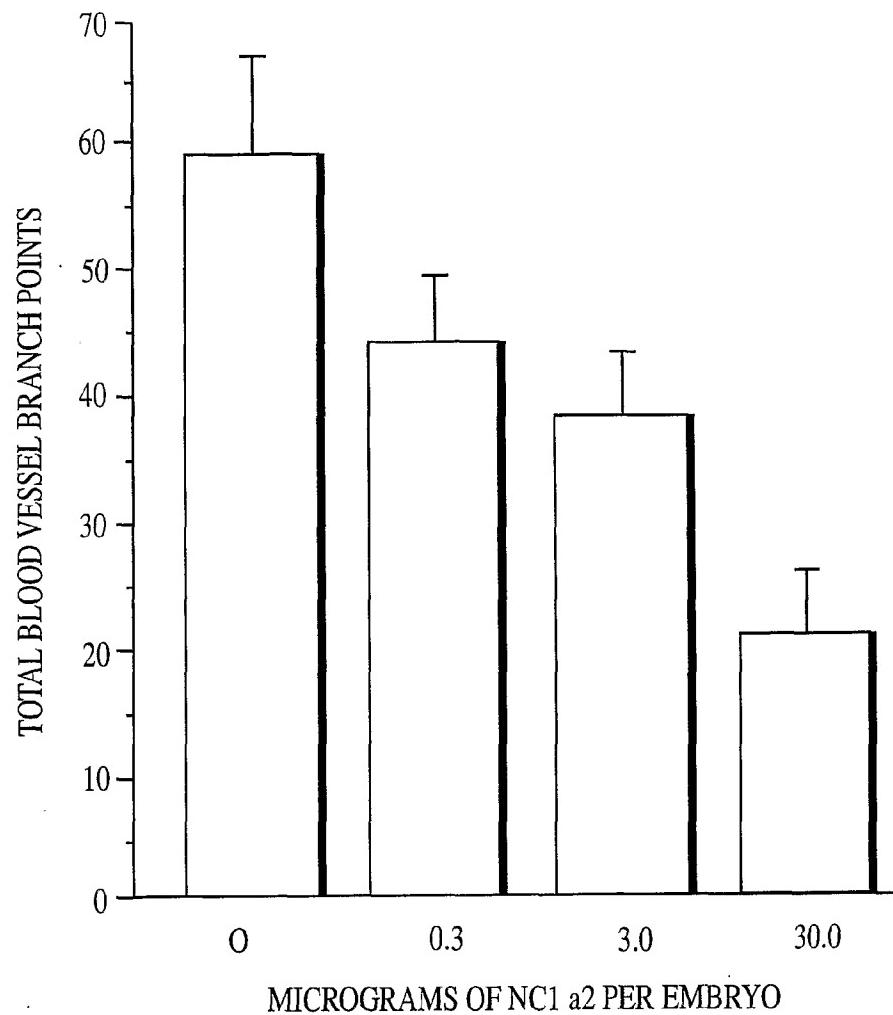


FIG. 7

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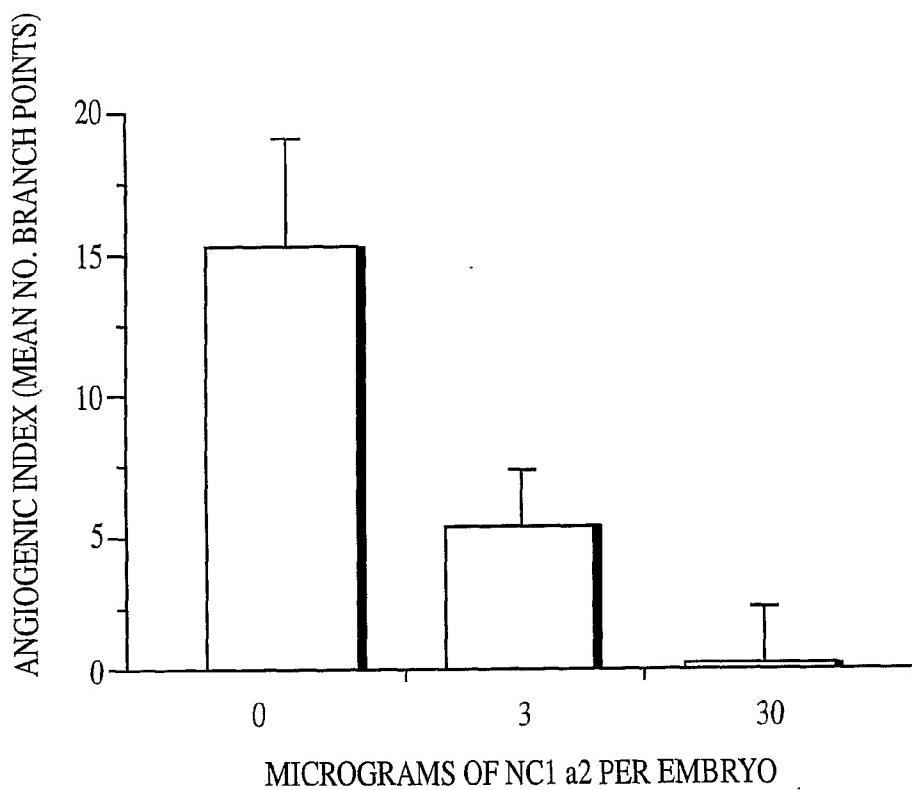


FIG. 8

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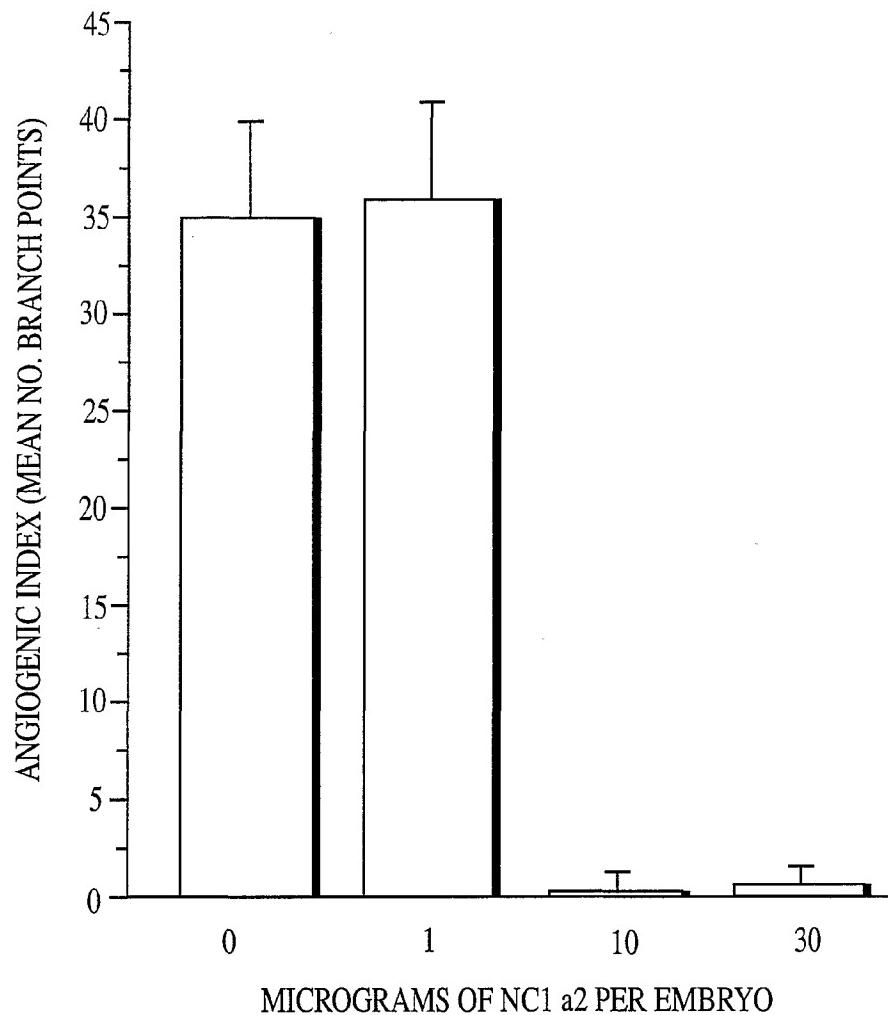


FIG. 9

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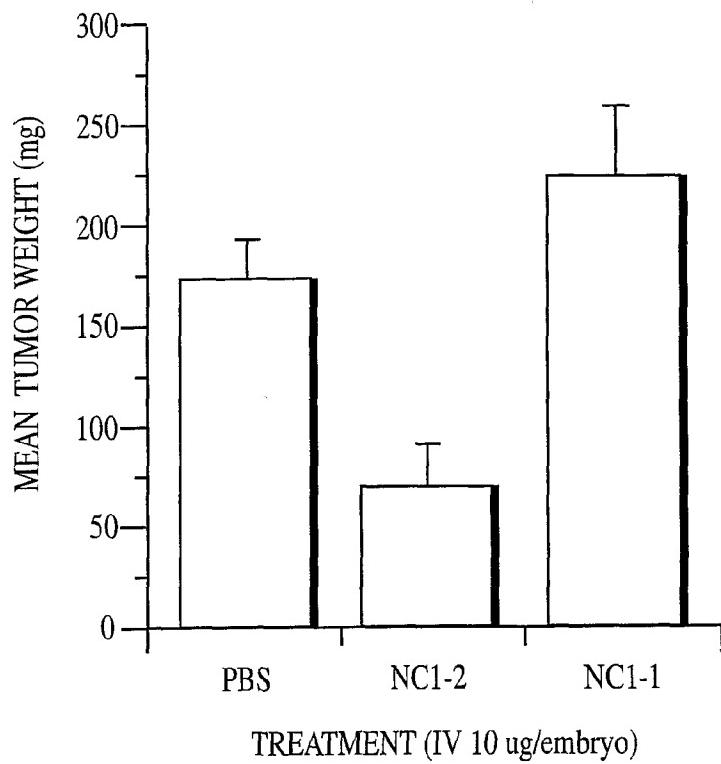


FIG. 10

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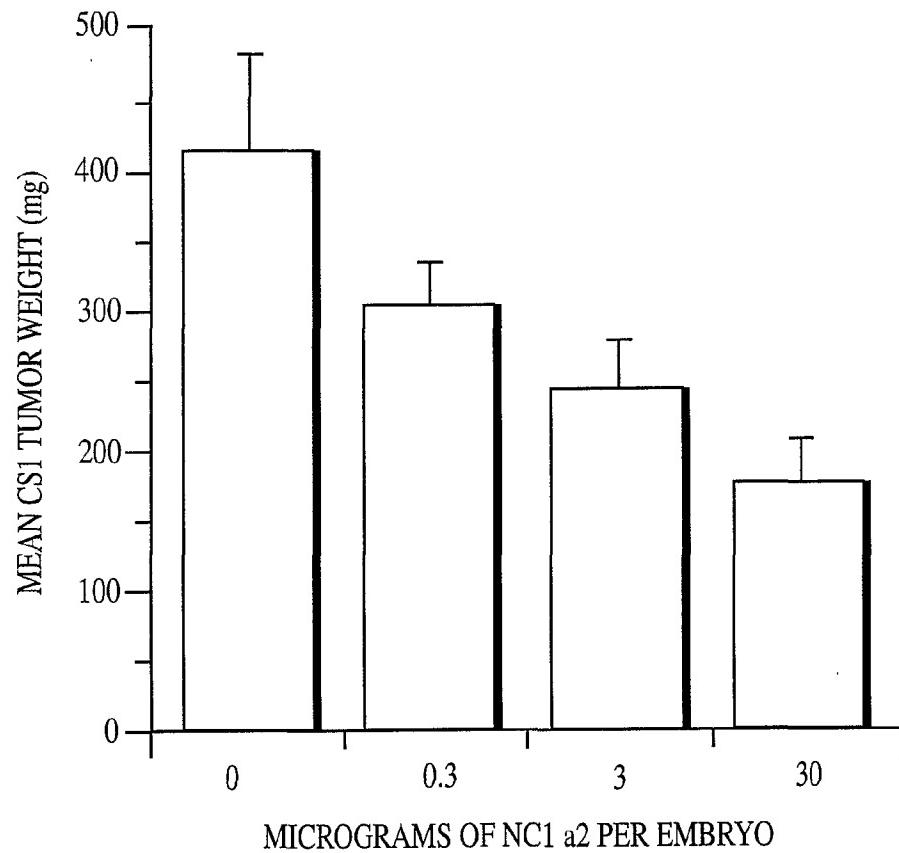


FIG. 11

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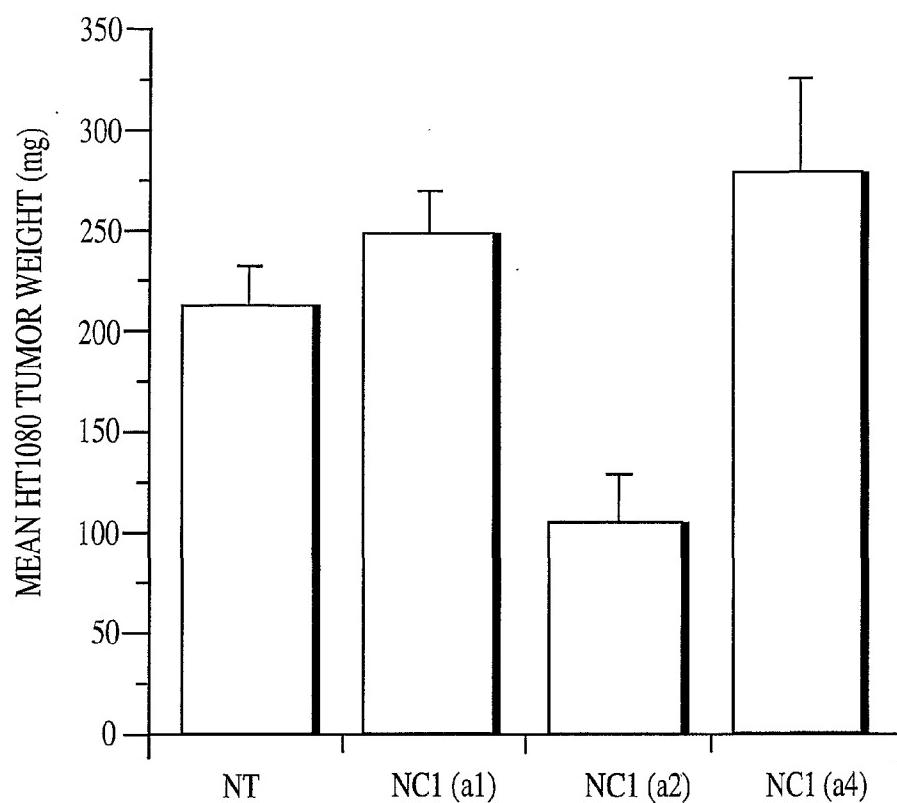


FIG. 12

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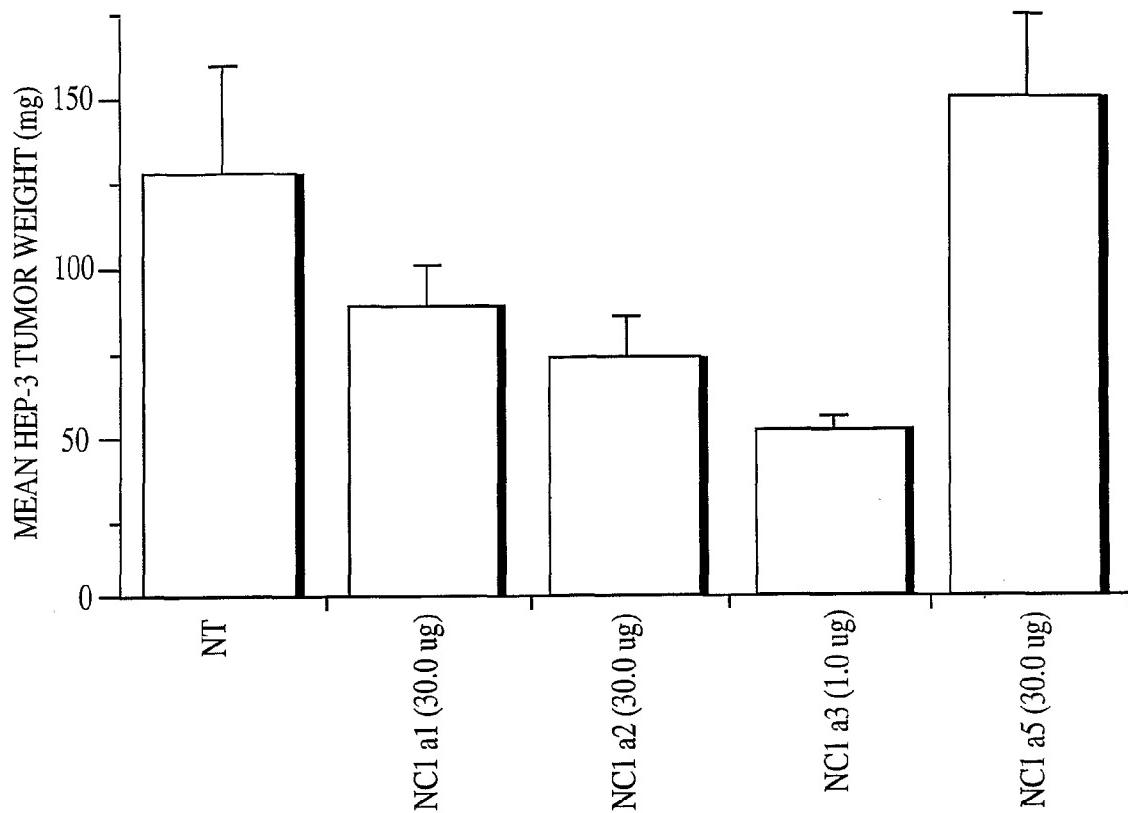


FIG. 13

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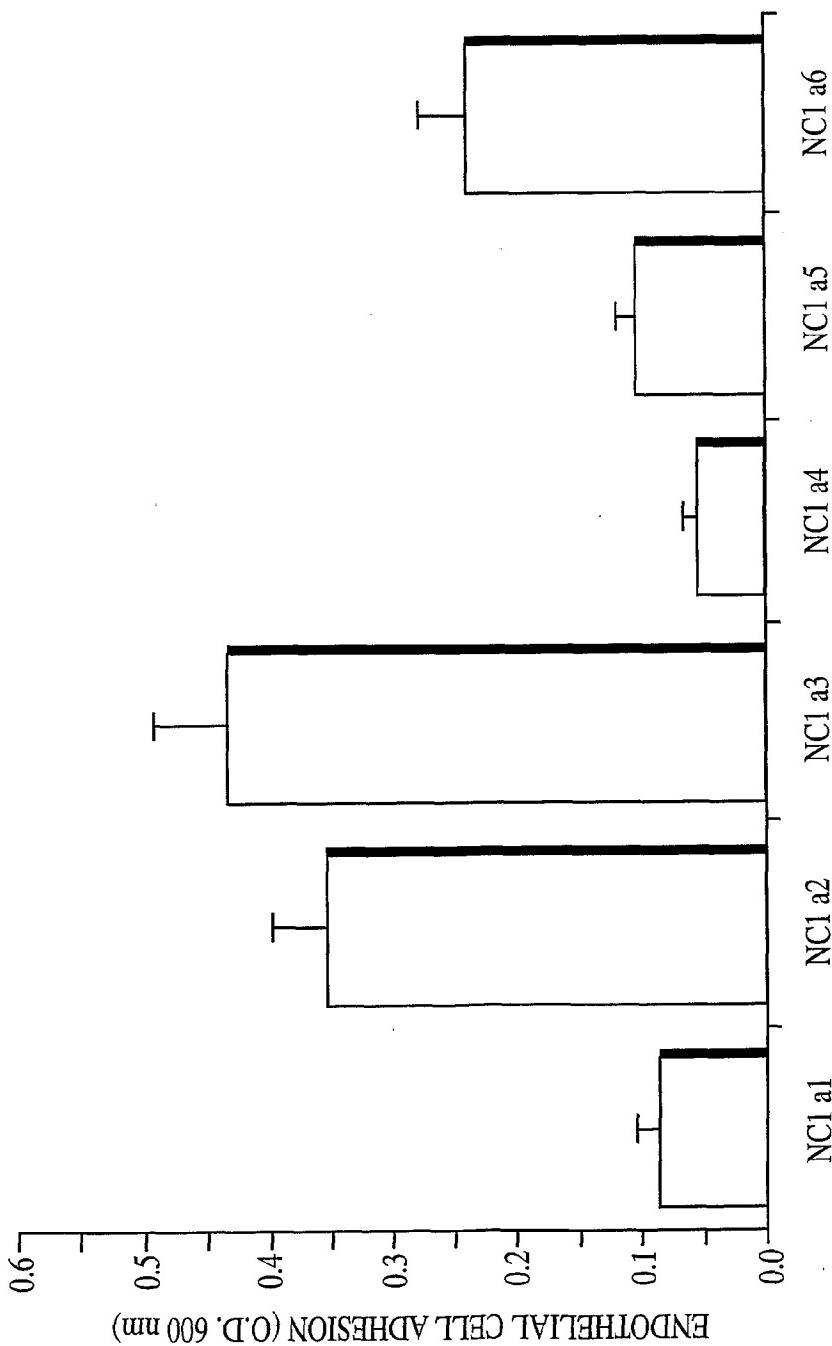


FIG. 14

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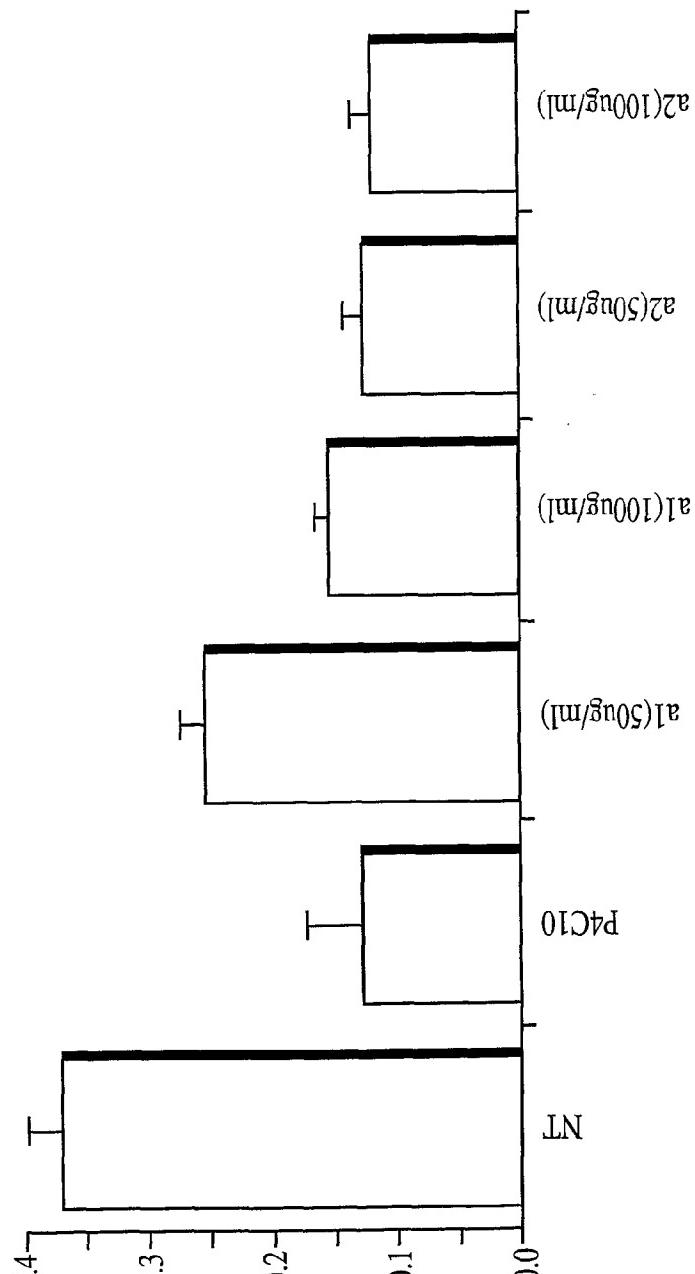


FIG. 15

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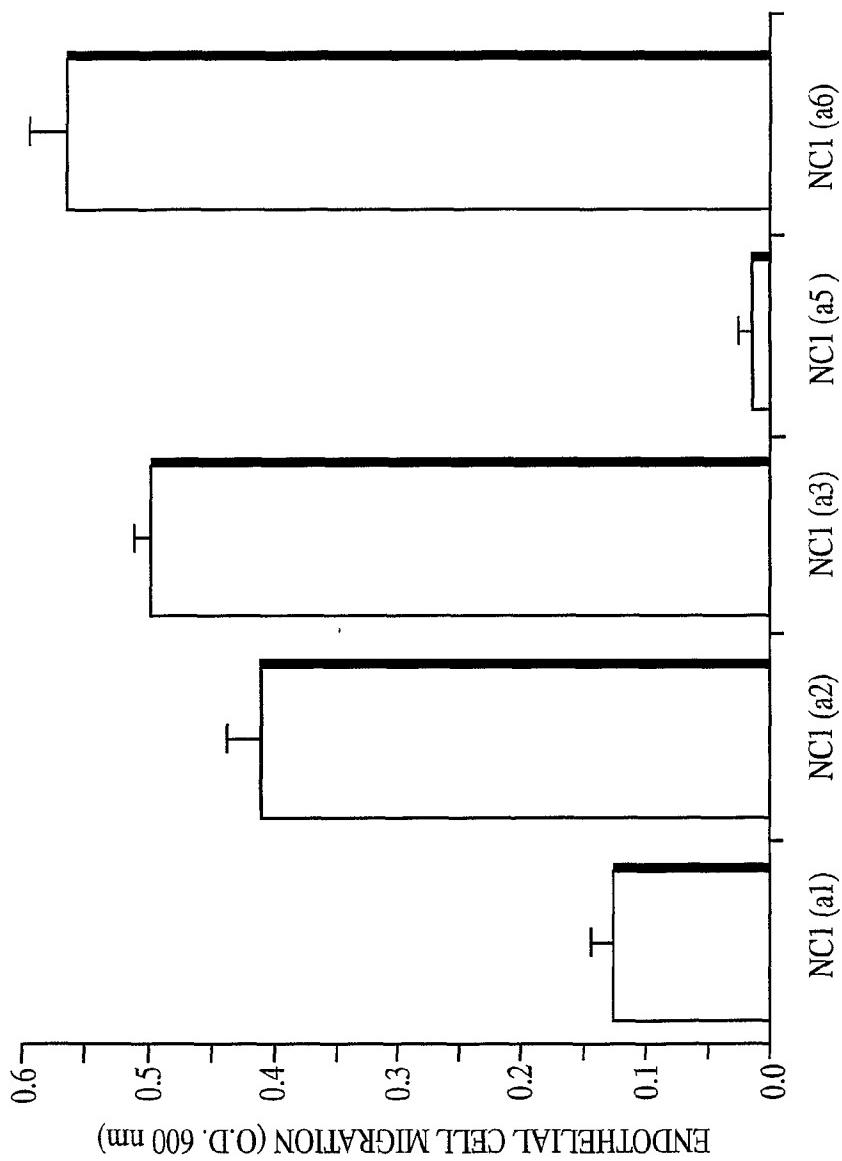


FIG. 16

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A. $\alpha 1$ (IV) NC1

900 910 920 930 940 950
 CTGCCGCCTGCCTGCCACTGAGGGTTCCCAGCACCATGAGGGCTGGATCTTCTTT
 M R A W I F F

960 970 980 990 1000 1010
 CTCCTTGCCCTGGCGGGAGGGCTCTGGCAGCCCCACTAGCCGACTACAAGGACGACGAT
 L L C L A G R A L A A P L A D Y K D D D

1020 1030 1040 1050 1060 1070
 GACAAGCTAGCATCTGTTGATCACGGCTTCCTGTGACCAAGGCATAAGTCAAACAAATAGAT
 D K L A S V D H G F L V T R H S Q T I D

1080 1090 1100 1110 1120 1130
 GACCCACAGTGTCCCTCTGGGACCAAAATTCTTACCAACGGGTACTCTTGCTCTACGTG
 D P Q C P S G T K I L Y H G Y S L L Y V

1140 1150 1160 1170 1180 1190
 CAAGGCAATGAACGGGCCATGGCCAGGACTTGGGCACGGCCGGCAGCTGCCTGCGCAAG
 Q G N E R A H G Q D L G T A G S C L R K

1200 1210 1220 1230 1240 1250
 TTCAGCACAATGCCCTCCTGTTCTGCAATATTAACAACGTGTGCAACTTGCATCACGA
 F S T M P F L F C N I N N V C N F A S R

1260 1270 1280 1290 1300 1310
 AATGACTACTCGTACTGGCTGTCCACCCCTGAGCCCAGTGTCAATGGCACCCATC
 N D Y S Y W L S T P E P M P M S M A P I

1320 1330 1340 1350 1360 1370
 ACGGGGGAAACATAAGACCATTATTAGTAGGTGTGCTGTGTGAGGCGCCTGCCATG
 T G E N I R P F I S R C A V C E A P A M

1380 1390 1400 1410 1420 1430
 GTGATGGCCGTGCACAGCCAGACCATTAGATCCCACCGTGCCCCAGCGGGTGGTCCTCG
 V M A V H S Q T I Q I P P C P S G W S S

1440 1450 1460 1470 1480 1490
 CTGTGGATCGGCTACTCTTGTGATGCACACCAGCGCTGGTGCAGAAGGCTCTGGCCAA
 L W I G Y S F V M H T S A G A E G S G Q

1500 1510 1520 1530 1540 1550
 GCCCTGGCGTCCCCCGGCTCCTGCCTGGAGGAGTTAGAAGTGCGCCATTGAGTGT

FIG. 17a

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A	L	A	S	P	G	S	C	L	E	E	F	R	S	A	P	F	I	E	C
---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---

1560 1570 1580 1590 1600 1610
 | | | | | |
 CACGGCCGTGGGACCTGCAATTACTACGCAAACGCTTACAGCTTTGGCTGCCACCATA
 H G R G T C N Y Y A N A Y S F W L A T I

 1620 1630 1640 1650 1660 1670
 | | | | | |
 GAGAGGAGCGAGATGTTCAAGAACGCCTACGCCGTCCACCTTGAAAGGCAGGGAGCTGCGC
 E R S E M F K K P T P S T L K A G E L R

 1680 1690 1700 1710 1720 1730
 | | | | | |
 ACGCACGTCAGCCGCTGCCAAGTCTGTATGAGAACATAATGAAGCCTGACTCAGCTA
 T H V S R C Q V C M R R T - -

 1740 1750 1760 1770 1780 1790
 | | | | | |
 CCGGGGGCCCTATTCTATAGTGTACCTAAATGCTAGAGCTCGCTGATCAGCCTCGACTG

FIG. 17a

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B. α2(IV) NC1

900	910	920	930	940	950	
CTGCCGCCTGCCTGCCACTGAGGGTTCCAGCACCATGAGGGCTGGATCTTCTTT						
						M R A W I F F
960	970	980	990	1000	1010	
CTCCTTGCTGGCCGGAGGGCTCTGGCAGCCCCACTAGCCGACTACAAGGACGACGAT						
						L L C L A G R A L A A P L A D Y K D D D
1020	1030	1040	1050	1060	1070	
GACAAGCTAGCGTCAGCATCGGCTACCTCTGGTGAAGCACAGCCAGACGGACCAGGAG						
						D K L A V S I G Y L L V K H S Q T D Q E
1080	1090	1100	1110	1120	1130	
CCCATGTGCCCGGTGGCATGAACAAACTCTGGAGTGGATACAGCCTGCTGTACTTCGAG						
						P M C P V G M N K L W S G Y S L L Y F E
1140	1150	1160	1170	1180	1190	
GGCCAGGAGAAGGCGCACAAACCAGGACCTGGGGCTGGCGGGCTCCTGCCCTGGCGCGGTTCA						
						G Q E K A H N Q D L G L A G S C L A R F
1200	1210	1220	1230	1240	1250	
AGCACCATGCCCTTCCTGTACTGCAACCCCTGGTGTCTGCTACTATGCCAGCCGAAC						
						S T M P F L Y C N P G D V C Y Y A S R N
1260	1270	1280	1290	1300	1310	
GACAAGTCCTACTGGCTCTCTACCACTGCGCCGCTGCCCATGATGCCGTGGCCGAGGAC						
						D K S Y W L S T T A P L P M M P V A E D
1320	1330	1340	1350	1360	1370	
GAGATCAAGCCCTACATCAGCCGCTGTTCTGTGTGAGGCCGCATGCCATCGCG						
						E I K P Y I S R C S V C E A P A I A I A
1380	1390	1400	1410	1420	1430	
GTCCACAGTCAGGATGTCTCCATCCACACTGCCCAGCTGGGGCGGAGTTGTGGATC						
						V H S Q D V S I P H C P A G W R S L W I
1440	1450	1460	1470	1480	1490	
GGATATTCCCTCCATGCACACGGGGGGAGACGAAGGCGGTGGCCAATCACTGGTG						
						G Y S F L M H T A A G D E G G G Q S L V
1500	1510	1520	1530	1540	1550	

FIG. 17b

TCACCGGGCAGCTGTCTAGAGGACTTCGCCACACCATTCAATCGAATGCAATGGAGGC
 S P G S C L E D F R A T P F I E C N G G
 1560 1570 1580 1590 1600 1610
 CGCGGCACCTGCCACTACTACGCCAACAAAGTACAGCTCTGGCTGACCACCATTCCCGAG
 R G T C H Y Y A N K Y S F W L T T I P E
 1620 1630 1640 1650 1660 1670
 CAGAGCTTCCAGGGCTCGCCCTCCGCCGACACGCTCAAGGCCGGCCTCATCCGCACACAC
 Q S F Q G S P S A D T L K A G L I R T H
 1680 1690 1700 1710 1720 1730
 ATCAGCCGCTGCCAGGTGTGCATGAAGAACCTGTGAGCCGGCGCGTGCCAGGGCCATT
 I S R C Q V C M K N L -
 1740 1750 1760 1770 1780 1790
 CTATAGTGTACCTAAATGCTAGAGCTCGCTGATCAGCCTCGACTGTGCCTTAGTTGC

FIG. 17b

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C. α 3(IV) NC1

900	910	920	930	940	950	
CTGCCGCCTGCCTGCCACTGAGGGTCCAGCACCATGAGGCCTGGATCTTCTTT						
						M R A W I F F
960	970	980	990	1000	1010	
CTCCTTGCCTGGCGGGGGCTCTGGCAGCCCCGCTAGCCGACTACAAGGACGACGAT						
						L L C L A G R A L A A P L A D Y K D D D
1020	1030	1040	1050	1060	1070	
GACAAACGTGGAGACAGTGGATCACCTGCAACCTGGACAAACGAGAGGGTTTGCTTCACC						
						D K R G D S G S P A T W T T R G F V F T
1080	1090	1100	1110	1120	1130	
CGACACAGTCAAACACAGCAATTCTTCATGTCCAGAGGGACAGTGCCACTCTACAGT						
						R H S Q T T A I P S C P E G T V P L Y S
1140	1150	1160	1170	1180	1190	
GGGTTTTCTTTCTTTGTACAAGGAAATCAACGAGCCCACGGACAAGACCTTGGAACT						
						G F S F L F V Q G N Q R A H G Q D L G T
1200	1210	1220	1230	1240	1250	
CTTGGCAGCTGCCTGCAGCGATTACCAATGCCATTCTTATTCTGCAATGTCAATGAT						
						L G S C L Q R F T T M P F L F C N V N D
1260	1270	1280	1290	1300	1310	
GTATGTAATTTGCATCTCGAAATGATTATTCATACTGGCTGTCAACACCCAGCTCTGATG						
						V C N F A S R N D Y S Y W L S T P A L M
1320	1330	1340	1350	1360	1370	
CCAATGAACATGGCTCCCATTACTGGCAGAGCCCTTGAGCCTTATATAAGCAGATGCACT						
						P M N M A P I T G R A L E P Y I S R C T
1380	1390	1400	1410	1420	1430	
GTTTGTGAAGGTCTGCATGCCATAGCCGTTCACAGCCAAACCACTGACATTCCCTCCA						
						V C E G P A I A I A V H S Q T T D I P P
1440	1450	1460	1470	1480	1490	
TGTCCCTCACGGCTGGATTCTCTCTGGAAAGGATTTCATTCAATCATGTTACAAGTGCA						
						C P H G W I S L W K G F S F I M F T S A
1500	1510	1520	1530	1540	1550	

FIG. 17c

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GGTTCTGAGGGCGCCGGGCAAGCACTGGCCTCCCCGGCTCCTGCCTGGAAGAATTCCGA
 G S E G A G Q A L A S P G S C L E E F R

1560 1570 1580 1590 1600 1610
 | | | | | |
 GCCAGCCCATTCTAGAACATGGAAAGAGGAACGTGCAACTACTATTCAAATTCCCTAC
 A S P F L E C H G R G T C N Y Y S N S Y

1620 1630 1640 1650 1660 1670
 | | | | | |
 AGTTTCTGGCTGGCTTCATTAAACCCAGAAAAAATGTTAGAAAGCTATTCCATCAACT
 S F W L A S L N P E R M F R K P I P S T

1680 1690 1700 1710 1720 1730
 | | | | | |
 GTGAAAGCTGGGAATTAGAAAAAATAATAAGTCGCTGTCAGGTGTGCATGAAGAAAAGA
 V K A G E L E K I I S R C Q V C M K K R

1740 1750 1760 1770 1780 1790
 | | | | | |
 CACTGAGGGCCCTATTCTATAGTGTACCTAAATGCTAGAGCTCGCTGATCAGCCTCGAC

H ~

FIG. 17c

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D. α4(IV)NC1

900	910	920	930	940	950	
CTGCCGCCTGCCTGCCACTGAGGGTCCAGCACCATGAGGGCCTGGATCTTCTTT						
						M R A W I F F
960	970	980	990	1000	1010	
CTCCTTGCCTGGCGGGAGGGCTCTGGCAGCCCCGCTAGCCGACTACAAGGACGACGAT						
						L L C L A G R A L A A P L A D Y K D D D
1020	1030	1040	1050	1060	1070	
GACAAGCCTGGATACTCGGTGGCTTCCTCCTGGTTCTCCACAGTCAGACGGACCAGGAG						
						D K P G Y L G G F L L V L H S Q T D Q E
1080	1090	1100	1110	1120	1130	
CCCACCTGCCCTGGGCATGCCAGGCTCTGGACTGGTATAGTCTGTTACCTGGAA						
						P T C P L G M P R L W T G Y S L L Y L E
1140	1150	1160	1170	1180	1190	
GGGCAAGAGAAAGCTCACAAATCAAGACCTTGGTCTGGCAGGGTCTTGCCTCCGTATTT						
						G Q E K A H N Q D L L G L A G S C L P V F
1200	1210	1220	1230	1240	1250	
AGCACGCTGCCCTTGCTACTGCAACATCCACCAGGTGTGCCACTATGCCAGAGAAC						
						S T L P F A Y C N I H Q V C H Y A Q R N
1260	1270	1280	1290	1300	1310	
GACAGATCCTACTGGCTGGCCAGCGCTGCGCCCTCCCATGATGCCACTCTGAAGAG						
						D R S Y W L A S A A P L P M M P L S E E
1320	1330	1340	1350	1360	1370	
GCGATCCGCCCTATGTCAGCCGCTGTGGTATGCGAGGCCCGGCCAGGGTGGCG						
						A I R P Y V S R C A V C E A P A Q A V A
1380	1390	1400	1410	1420	1430	
GTGCACAGCCAGGACCAGTCATCCCCCATGTCGGCAGACCTGGAGGAGCCTGGATC						
						V H S Q D Q S I P P C P Q T W R S L W I
1440	1450	1460	1470	1480	1490	
GGGTATTCTGATGCACACAGGAGCTGGGACCAAGGAGGAGGGCAGGCCCTATG						
						G Y S F L M H T G A G D Q G G G Q A L M
1500	1510	1520	1530	1540	1550	

FIG. 17d

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TCACCTGGCAGCTGCCTGGAAGATTCAGAGCAGCACCATTCCTGAATGCCAGGGCCGG
 S P G S C L E D F R A A P F L E C Q G R
 1560 1570 1580 1590 1600 1610
 CAGGGAACTTGCCACTTTTCGCAAATAAGTATAGCTTCTGGCTCACAACGGTGAAAGCA
 Q G T C H F F A N K Y S F W L T T V K A
 1620 1630 1640 1650 1660 1670
 GACTTGCAGTTTCCTCTGCTCCAGCACCAGACACCTTAAAAGAAAGCCAGGGCCCAACGC
 D L Q F S S A P A P D T L K E S Q A Q R
 1680 1690 1700 1710 1720 1730
 CAGAAAATCAGCCGGTGCCAGGTCTGCGTGAAGTATAGCTAGGGCCCTATTCTATAGTG
 Q K I S R C Q V C V K Y S -
 1740 1750 1760 1770 1780 1790
 TCACCTAAATGCTAGAGCTCGCTGATCAGCCTCGACTGTGCCTTAGTTGCCAGCCATC

FIG. 17d

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E. α 5(IV) NC1

900	910	920	930	940	950	
CTGCCGCCTGCCTGCCACTGAGGGTTCCCAGCACCATGAGGGCCTGGATCTCTTT						
						M R A W I F F
960	970	980	990	1000	1010	
CTCCTTGCCTGGCCGGGAGGGCTCTGGCAGCCCCGCTAGCTGACTACAAGGACGACGAT						
L L C L A G R A L A A P L A D Y K D D D						
1020	1030	1040	1050	1060	1070	
GACAAAGGTCCCCCTGGAACCTCCTCTGTCACATGGATTCTTCTIATTACACGCCACAGC						
D K G P P G T S S V A H G F L I T R H S						
1080	1090	1100	1110	1120	1130	
CAGACAAACGGATGCACCAATGCCAACAGGAAACACTCAGGTCTATGAAGGCTTTCT						
Q T T D A P Q C P Q G T L Q V Y E G F S						
1140	1150	1160	1170	1180	1190	
CTCCTGTATGTACAAGGAAATAAAGAGGCCACGGTCAAGACTTGGGGACGGCTGGCAGC						
L L Y V Q G N K R A H G Q D L G T A G S						
1200	1210	1220	1230	1240	1250	
TGCCTTCGTCGCTTTAGTACCATGCCTTCATGTTCTGCAACATCAATAATGTTGCAAC						
C L R R F S T M P F M F C N I N N V C N						
1260	1270	1280	1290	1300	1310	
TTTGCTTCAAGAAATGACTATTCTACTGGCTCTACCCCAGAGGCCATGCCATGAGC						
F A S R N D Y S Y W L S T P E P M P M S						
1320	1330	1340	1350	1360	1370	
ATGCAACCCCTAAAGGCCAGAGCATCCAGCCATTCAATTAGTCGATGTGCAGTATGTGAA						
M Q P L K G Q S I Q P F I S R C A V C E						
1380	1390	1400	1410	1420	1430	
GCTCCAGCTGTGGTGTAGCGAGTCACAGTCAGACGATCCAGATTCCCCATTGTCCTCAG						
A P A V V I A V H S Q T I Q I P H C P Q						
1440	1450	1460	1470	1480	1490	
GGATGGGATTCTGTGGATTGGTATTCCCTCATGATGCATAAAGTGCAGGGGCAGAA						
G W D S L W I G Y S F M M H T S A G A E						
1500	1510	1520	1530	1540	1550	

FIG. 17e

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GGCTCAGGTCAAGCCCTAGCCTCCCCTGGTTCTGCTTGGAAAGAGTTTCGTTCAGCTCCC
 G S G Q A L A S P G S C L E E F R S A P
 1560 1570 1580 1590 1600 1610
 TTCATCGAATGTCATGGGAGGGTACCTGTAACTACTATGCCAACCTACAGCTTTGG
 F I E C H G R G T C N Y Y A N S Y S F W
 1620 1630 1640 1650 1660 1670
 CTGGCAACTGTAGATGTGTCAGACATGTTCAAGTAAACCTCAGTCAGAAACGCTGAAAGCA
 L A T V D V S D M F S K P Q S E T L K A
 1680 1690 1700 1710 1720 1730
 GGAGACTTGAGGACACGAATTAGCCGATGTCAAGTGTGCATGAAGAGGGACATAACGCGGC
 G D L R T R I S R C Q V C M K R T -
 1740 1750 1760 1770 1780 1790
 CGCTCGAGCATGCATCTAGAGGGCCCTATTCTATAGTGTCACCTAAATGCTAGAGCTCGC

FIG. 17e

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F. $\alpha 6$ (IV) NCL

900	910	920	930	940	950	
CTGCCGCCTGCCTGCCACTGAGGGTTCCAGCACCATGAGGGCTGGATCTTCTTT						
M	R	A	W	I	F	F
960	970	980	990	1000	1010	
CTCCTTGCGCTGGCCGGAGGGCTCTGGCAGCCCCACTAGCCGACTACAAGGACGACGAT						
L	L	C	L	A	G	R
A	R	A	L	A	P	L
					D	Y
					K	D
					D	D
1020	1030	1040	1050	1060	1070	
GACAAGCTAGCGAGCATGAGAGTGGGCTACACGTTGGTAAGCACAGCCAGTCGGAACAG						
D	K	L	A	S	M	R
					V	G
					Y	T
					L	V
					K	H
					S	Q
					S	E
					Q	Q
1080	1090	1100	1110	1120	1130	
GTGCCCGGTGTCCCATGGGATGAGCCAGCTGTGGTGGGTACAGCTTACTGTTGTG						
V	P	P	C	P	I	G
					M	M
					S	Q
					L	L
					F	V
1140	1150	1160	1170	1180	1190	
GAGGGGCAAGAGAAAGCCCACAACCAGGACCTGGCTTGCTGGCTCCTGTCTGCCCGC						
E	G	Q	E	K	A	H
					N	N
					Q	D
					L	L
					G	F
					A	A
					G	S
					S	C
					L	P
					R	R
1200	1210	1220	1230	1240	1250	
TTCAGCACCATGCCCTCATCTACTGCAACATCAACGAGGTGTGCCACTATGCCAGGCGC						
F	S	T	M	P	F	I
					Y	Y
					C	N
					I	I
					N	N
					E	E
					V	V
					C	C
					H	Y
					Y	A
					A	R
					R	R
1260	1270	1280	1290	1300	1310	
AATGATAAACTTACTGGCTCTCACTACCAGGCCCTATCCCCATGATGCCGTAGCCAG						
N	D	K	S	Y	W	L
					S	S
					T	T
					A	A
					P	P
					M	M
					P	V
					S	Q
					Q	Q
					A	I
1320	1330	1340	1350	1360	1370	
ACCCAGATTCCCCAGTACATCAGCCGCTGCTGTGTGAGGCACCCCTCGCAAGCCATT						
T	Q	I	P	Q	Y	I
					S	R
					R	C
					S	S
					V	C
					C	E
					A	A
					P	P
					S	Q
					Q	A
					A	I
1380	1390	1400	1410	1420	1430	
GCTGTGCACAGCCAGGACATACCATCCCGCAGTGCCCCCTGGCTGGCGAGCCTCTGG						
A	V	H	S	Q	D	I
						T
						I
						P
						Q
						C
						P
						L
						G
						W
						R
						S
						L
1440	1450	1460	1470	1480	1490	
ATTGGGTACTTTCTCATGCACACTGCCGCTGGTGCCGAGGGTGAGGCCAGTCCCTG						
I	G	Y	S	F	L	M
						H
						T
						A
						G
						A
						E
						G
						G
						Q
						S
						L
1500	1510	1520	1530	1540	1550	

FIG. 17f

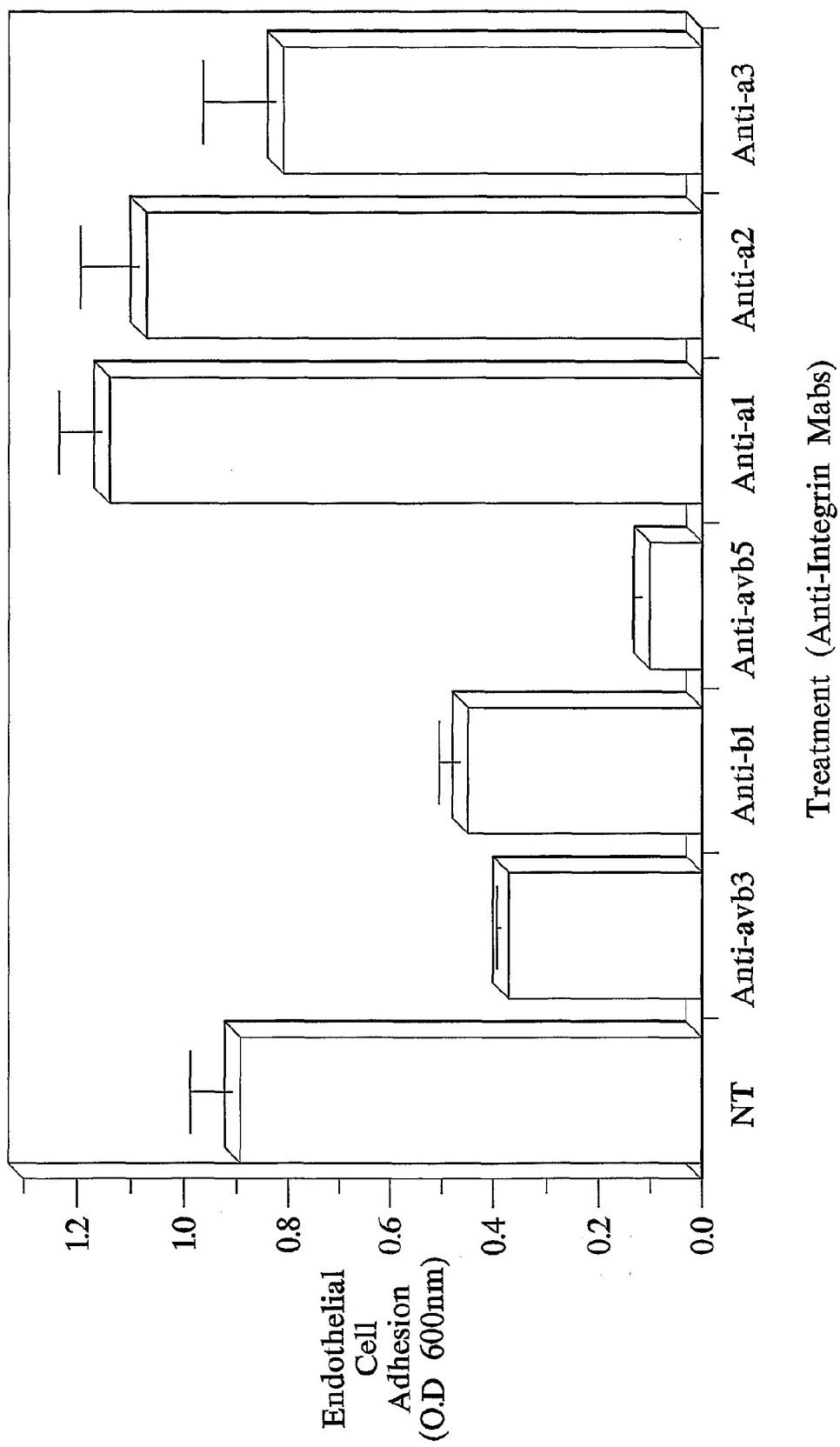
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GTCTCACCTGGCTCCTGCCTAGAGGACTTTGGGCCACTCCTTCATCGAATGCAGTGGT
 V S P G S C L E D F R A T P F I E C S G
 1560 1570 1580 1590 1600 1610
 GCCCGAGGCACCTGCCACTACTTGAAACAAGTACAGTTCTGGTTGACCACAGTGGAG
 A R G T C H Y F A N K Y S F W L T T V E
 1620 1630 1640 1650 1660 1670
 GAGAGGCAGCAGTTGGGGAGTTGCCTGTCTGAAACGCTGAAAGCTGGCAGCTCCAC
 E R Q Q F G E L P V S E T L K A G Q L H
 1680 1690 1700 1710 1720 1730
 ACTCGAGTCAGTCGCTGCCAGGTGTATGAAAAGCCTGTAGGGTGGCACCTGCCACGGG
 T R V S R C Q V C M K S L -
 1740 1750 1760 1770 1780 1790
 CCCTATTCTATAGTGTACCTAAATGCTAGAGCTCGCTGATCAGCCTCGACTGTGCCTTC

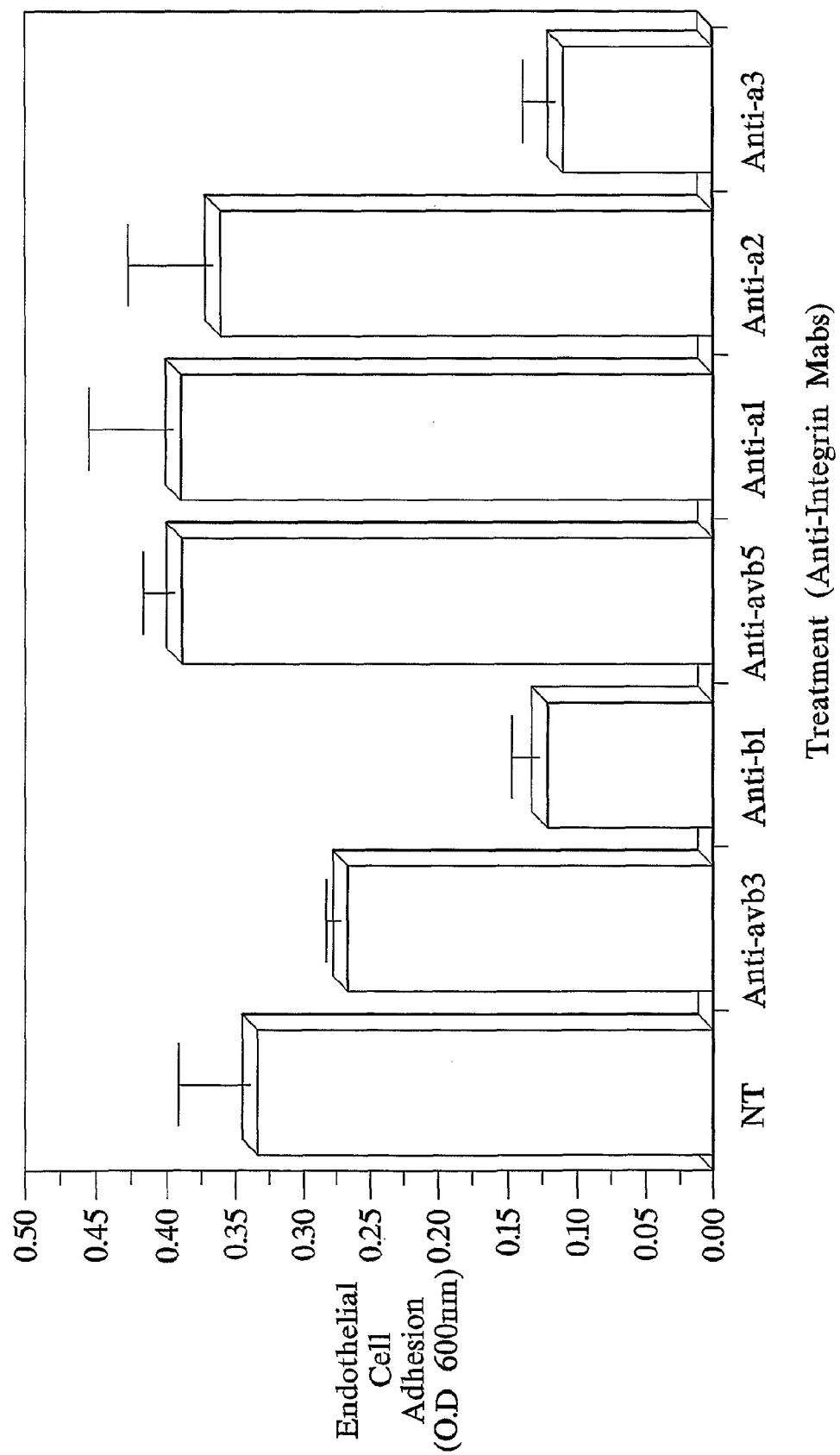
FIG. 17f

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FIG. 18



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FIG. 19

SEQUENCE LISTING

<110> Biostratum, Inc. et al.

<120> Methods to Inhibit Angiogenesis and Tumor Growth

<130> 94525K2

<140> To be assigned

<141> Herewith

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		Met Arg Ala Trp Ile	
		1	5

ttc ttt ctc ctt tgc ctg gcc ggg agg gct ctg gca gcc cca cta gcc	Phe Phe Leu Leu Cys Leu Ala Gly Arg Ala Leu Ala Ala Pro Leu Ala	10	15	20	102
---	---	----	----	----	-----

gac tac aag gac gac gat gac aag cta gca tct gtt gat cac ggc ttc	Asp Tyr Lys Asp Asp Asp Asp Lys Leu Ala Ser Val Asp His Gly Phe	25	30	35	150
---	---	----	----	----	-----

ctt gtg acc agg cat agt caa aca ata gat gac cca cag tgt cct tct	Leu Val Thr Arg His Ser Gln Thr Ile Asp Asp Pro Gln Cys Pro Ser	40	45	50	198
---	---	----	----	----	-----

ggg acc aaa att ctt tac cac ggg tac tct ttg ctc tac gtg caa ggc	Gly Thr Lys Ile Leu Tyr His Gly Tyr Ser Leu Leu Tyr Val Gln Gly	55	60	65	246
---	---	----	----	----	-----

aat gaa cgg gcc cat ggc cag gac ttg ggc acg gcc ggc agc tgc ctg	Asn Glu Arg Ala His Gly Gln Asp Leu Gly Thr Ala Gly Ser Cys Leu	70	75	80	85	294
---	---	----	----	----	----	-----

cgc aag ttc agc aca atg ccc ttc ctg ttc tgc aat att aac aac gtg	Arg Lys Phe Ser Thr Met Pro Phe Leu Phe Cys Asn Ile Asn Asn Val	90	95	100	342
---	---	----	----	-----	-----

tgc aac ttt gca tca cga aat gac tac tcg tac tgg ctg tcc acc cct	Cys Asn Phe Ala Ser Arg Asn Asp Tyr Ser Tyr Trp Leu Ser Thr Pro	105	110	115	390
---	---	-----	-----	-----	-----

gag ccc atg ccc atg tca atg gca ccc atc acg ggg gaa aac ata aga 438
 Glu Pro Met Pro Met Ser Met Ala Pro Ile Thr Gly Glu Asn Ile Arg
 120 125 130

 cca ttt att agt agg tgt gct gtg tgt gag gcg cct gcc atg gtg atg 486
 Pro Phe Ile Ser Arg Cys Ala Val Cys Glu Ala Pro Ala Met Val Met
 135 140 145

 gcc gtg cac agc cag acc att cag atc cca ccg tgc ccc agc ggg tgg 534
 Ala Val His Ser Gln Thr Ile Gln Ile Pro Pro Cys Pro Ser Gly Trp
 150 155 160 165

 tcc tcg ctg tgg atc ggc tac tct ttt gtg atg cac acc agc gct ggt 582
 Ser Ser Leu Trp Ile Gly Tyr Ser Phe Val Met His Thr Ser Ala Gly
 170 175 180

 gca gaa ggc tct ggc caa gcc ctg gcg tcc ccc ggc tcc tgc ctg gag 630
 Ala Glu Gly Ser Gly Gln Ala Leu Ala Ser Pro Gly Ser Cys Leu Glu
 185 190 195

 gag ttt aga agt gcg cca ttc atc gag tgt cac ggc cgt ggg acc tgc 678
 Glu Phe Arg Ser Ala Pro Phe Ile Glu Cys His Gly Arg Gly Thr Cys
 200 205 210

 aat tac tac gca aac qct tac agc ttt tgg ctc gcc acc ata gag agg 726
 Asn Tyr Tyr Ala Asn Ala Tyr Ser Phe Trp Leu Ala Thr Ile Glu Arg
 215 220 225

 agc gag atg ttc aag aag cct acg ccg tcc acc ttg aag gca ggg gag 774
 Ser Glu Met Phe Lys Lys Pro Thr Pro Ser Thr Leu Lys Ala Gly Glu
 230 235 240 245

 ctg cgc acg cac qtc agc cgc tgc caa gtc tgt atg aga aga aca 819
 Leu Arg Thr His Val Ser Arg Cys Gln Val Cys Met Arg Arg Thr
 250 255 260

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 20 25 30

 Val Asp His Gly Phe Leu Val Thr Arg His Ser Gln Thr Ile Asp Asp
 35 40 45

 Pro Gln Cys Pro Ser Gly Thr Lys Ile Leu Tyr His Gly Tyr Ser Leu

3

50 55 60

Leu Tyr Val Gln Gly Asn Glu Arg Ala His Gly Gln Asp Leu Gly Thr
65 70 75 80

Ala Gly Ser Cys Leu Arg Lys Phe Ser Thr Met Pro Phe Leu Phe Cys
 85 90 95

Trp Leu Ser Thr Pro Glu Pro Met Pro Met Ser Met Ala Pro Ile Thr
115 120 125

Gly Glu Asn Ile Arg Pro Phe Ile Ser Arg Cys Ala Val Cys Glu Ala
 130 135 140

Pro Ala Met Val Met Ala Val His Ser Gln Thr Ile Gln Ile Pro Pro
145 150 155 160

Cys Pro Ser Gly Trp Ser Ser Leu Trp Ile Gly Tyr Ser Phe Val Met
 165 170 175

His Thr Ser Ala Gly Ala Glu Gly Ser Gly Gln Ala Leu Ala Ser Pro
 180 185 190

Gly Ser Cys Leu Glu Glu Phe Arg Ser Ala Pro Phe Ile Glu Cys His
 195 200 205

Gly Arg Gly Thr Cys Asn Tyr Tyr Ala Asn Ala Tyr Ser Phe Trp Leu
210 215 220

Ala Thr Ile Glu Arg Ser Glu Met Phe Lys Lys Pro Thr Pro Ser Thr
 225 230 235 240

Leu Lys Ala Gly Glu Leu Arg Thr His Val Ser Arg Cys Gln Val Cys
245 250 255

Met Arg Arg Thr
260

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Met Arg Ala Trp Ile
1 5

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Phe Phe Leu Leu Cys Leu Ala Gly Arg Ala Leu Ala Ala Pro Leu Ala		
10	15	20
gac tac aag gac gac gat gac aag cta gcc gtc agc atc ggc tac ctc		150
Asp Tyr Lys Asp Asp Asp Lys Leu Ala Val Ser Ile Gly Tyr Leu		
25	30	35
ctg gtg aag cac agc cag acg gac cag gag ccc atg tgc ccg gtg ggc		198
Leu Val Lys His Ser Gln Thr Asp Gln Glu Pro Met Cys Pro Val Gly		
40	45	50
atg aac aaa ctc tgg agt gga tac agc ctg ctg tac ttc gag ggc cag		246
Met Asn Lys Leu Trp Ser Gly Tyr Ser Leu Leu Tyr Phe Glu Gly Gln		
55	60	65
gag aag gcg cac aac cag gac ctg ggg ctg gcg ggc tcc tgc ctg gcg		294
Glu Lys Ala His Asn Gln Asp Leu Gly Leu Ala Gly Ser Cys Leu Ala		
70	75	80
ccg ttc agc acc atg ccc ttc ctg tac tgc aac cct ggt gat gtc tgc		342
Arg Phe Ser Thr Met Pro Phe Leu Tyr Cys Asn Pro Gly Asp Val Cys		
90	95	100
tac tat gcc agc cgg aac gac aag tcc tac tgg ctc tct acc act gcg		390
Tyr Tyr Ala Ser Arg Asn Asp Lys Ser Tyr Trp Leu Ser Thr Thr Ala		
105	110	115
ccg ctg ccc atg atg ccc gtg gcc gag gac gag atc aag ccc tac atc		438
Pro Leu Pro Met Met Pro Val Ala Glu Asp Glu Ile Lys Pro Tyr Ile		
120	125	130
agc cgc tgt tct gtg tgt gag gcc ccc atc gac atc gac atc gac gtc cac		486
Ser Arg Cys Ser Val Cys Glu Ala Pro Ala Ile Ala Ile Ala Val His		
135	140	145
agt cag gat gtc tcc atc cca cac tgc cca gct ggg tgg cgg agt ttg		534
Ser Gln Asp Val Ser Ile Pro His Cys Pro Ala Gly Trp Arg Ser Leu		
150	155	160
165		
tgg atc gga tat tcc ttc ctc atg cac acg gcg gcg gga gac gaa ggc		582
Trp Ile Gly Tyr Ser Phe Leu Met His Thr Ala Ala Gly Asp Glu Gly		
170	175	180
ggt ggc caa tca ctg gtg tca ccc ggc agc tgt cta gag gac ttc cgc		630
Gly Gly Gln Ser Leu Val Ser Pro Gly Ser Cys Leu Glu Asp Phe Arg		
185	190	195
gcc aca cca ttc atc gaa tgc aat gga ggc cgc ggc acc tgc cac tac		678
Ala Thr Pro Phe Ile Glu Cys Asn Gly Gly Arg Gly Thr Cys His Tyr		
200	205	210
tac gcc aac aag tac agc ttc tgg ctg acc acc att ccc gag cag agc		726
Tyr Ala Asn Lys Tyr Ser Phe Trp Leu Thr Thr Ile Pro Glu Gln Ser		
215	220	225
ttc cag ggc tcg ccc tcc gcc gac acg ctc aag gcc ggc ctc atc cgc		774
Phe Gln Gly Ser Pro Ser Ala Asp Thr Leu Lys Ala Gly Leu Ile Arg		

230	235	240	245												
aca cac atc agc cgc tgc cag gtg tgc atg aag aac ctg tgagccggcg 823															
Thr	His	Ile	Ser	Arg	Cys	Gln	Val	Cys	Met	Lys	Asn	Leu			
				250	255										
cgtgccagg ccctattcta tagtgtcacc taaatgctag agctcgctga tcagcctcga 883															
ctgtgccttc tagttgc 900															
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Ala	Ala	Pro	Leu	Ala	Asp	Tyr	Lys	Asp	Asp	Asp	Lys	Leu	Ala	Val	
			20				25						30		
Ser	Ile	Gly	Tyr	Leu	Leu	Val	Lys	His	Ser	Gln	Thr	Asp	Gln	Glu	Pro
35					40						45				
Met	Cys	Pro	Val	Gly	Met	Asn	Lys	Leu	Trp	Ser	Gly	Tyr	Ser	Leu	Leu
50					55				60						
Tyr	Phe	Glu	Gly	Gln	Glu	Lys	Ala	His	Asn	Gln	Asp	Leu	Gly	Leu	Ala
65					70				75				80		
Gly	Ser	Cys	Leu	Ala	Arg	Phe	Ser	Thr	Met	Pro	Phe	Leu	Tyr	Cys	Asn
					85				90				95		
Pro	Gly	Asp	Val	Cys	Tyr	Tyr	Ala	Ser	Arg	Asn	Asp	Lys	Ser	Tyr	Trp
					100			105				110			
Leu	Ser	Thr	Thr	Ala	Pro	Leu	Pro	Met	Met	Pro	Val	Ala	Glu	Asp	Glu
					115			120				125			
Ile	Lys	Pro	Tyr	Ile	Ser	Arg	Cys	Ser	Val	Cys	Glu	Ala	Pro	Ala	Ile
130					135					140					
Ala	Ile	Ala	Val	His	Ser	Gln	Asp	Val	Ser	Ile	Pro	His	Cys	Pro	Ala
145					150					155			160		
Gly	Trp	Arg	Ser	Leu	Trp	Ile	Gly	Tyr	Ser	Phe	Leu	Met	His	Thr	Ala
					165				170			175			
Ala	Gly	Asp	Glu	Gly	Gly	Gln	Ser	Leu	Val	Ser	Pro	Gly	Ser	Cys	
						180		185				190			
Leu	Glu	Asp	Phe	Arg	Ala	Thr	Pro	Phe	Ile	Glu	Cys	Asn	Gly	Gly	Arg
					195			200				205			
Gly	Thr	Cys	His	Tyr	Tyr	Ala	Asn	Lys	Tyr	Ser	Phe	Trp	Leu	Thr	Thr

210	215	220
-----	-----	-----

Ile Pro Glu Gln Ser Phe Gln Gly Ser Pro Ser Ala Asp Thr Leu Lys		
225	230	235
		240

Ala Gly Leu Ile Arg Thr His Ile Ser Arg Cys Gln Val Cys Met Lys		
245	250	255

Asn Leu

<210> 5

<211> 900

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Met Arg Ala Trp Ile	
1	5

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Phe Phe Leu Leu Cys Leu Ala Gly Arg Ala Leu Ala Ala Pro Leu Ala	
10	15
	20

gac tac aag gac gac gat gac aaa cgt gga gac agt gga tca cct gca	150
Asp Tyr Lys Asp Asp Asp Lys Arg Gly Asp Ser Gly Ser Pro Ala	
25	30
	35

acc tgg aca acg aga ggc ttt gtc ttc acc cga cac agt caa acc aca	198
Thr Trp Thr Thr Arg Gly Phe Val Phe Thr Arg His Ser Gln Thr Thr	
40	45
	50

gca att cct tca tgt cca gag ggg aca gtg cca ctc tac agt ggg ttt	246
Ala Ile Pro Ser Cys Pro Glu Gly Thr Val Pro Leu Tyr Ser Gly Phe	
55	60
	65

tct ttt ctt ttt gta caa gga aat caa cga gcc cac gga caa gac ctt	294
Ser Phe Leu Phe Val Gln Gly Asn Gln Arg Ala His Gly Gln Asp Leu	
70	75
	80
	85

gga act ctt ggc agc tgc ctg cag cga ttt acc aca atg cca ttc tta	342
Gly Thr Leu Gly Ser Cys Leu Gln Arg Phe Thr Thr Met Pro Phe Leu	
90	95
	100

ttc tgc aat gtc aat gat gta tgt aat ttt gca tct cga aat gat tat	390
Phe Cys Asn Val Asn Asp Val Cys Asn Phe Ala Ser Arg Asn Asp Tyr	
105	110
	115

tca tac tgg ctg tca aca cca gct ctg atg cca atg aac atg gct ccc	438
Ser Tyr Trp Leu Ser Thr Pro Ala Leu Met Pro Met Asn Met Ala Pro	
120	125
	130

att act ggc aga gcc ctt gag cct tat ata agc aga tgc act gtt tgt Ile Thr Gly Arg Ala Leu Glu Pro Tyr Ile Ser Arg Cys Thr Val Cys 135 140 145	486
gaa ggt cct gcg atc gcc ata gcc gtt cac agc caa acc act gac att Glu Gly Pro Ala Ile Ala Ile Ala Val His Ser Gln Thr Thr Asp Ile 150 155 160 165	534
cct cca tgt cct cac ggc tgg att tct ctc tgg aaa gga ttt tca ttc Pro Pro Cys Pro His Gly Trp Ile Ser Leu Trp Lys Gly Phe Ser Phe 170 175 180	582
atc atg ttc aca agt gca ggt tct gag ggc gcc ggg caa gca ctg gcc Ile Met Phe Thr Ser Ala Gly Ser Glu Gly Ala Gly Gln Ala Leu Ala 185 190 195	630
tcc ccc ggc tcc tgc ctg gaa gaa ttc cga gcc agc cca ttt cta gaa Ser Pro Gly Ser Cys Leu Glu Glu Phe Arg Ala Ser Pro Phe Leu Glu 200 205 210	678
tgt cat gga aga gga acg tgc aac tac tat tca aat tcc tac agt ttc Cys His Gly Arg Gly Thr Cys Asn Tyr Tyr Ser Asn Ser Tyr Ser Phe 215 220 225	726
tgg ctg gct tca tta aac cca gaa aga atg ttc aga aag cct att cca Trp Leu Ala Ser Leu Asn Pro Glu Arg Met Phe Arg Lys Pro Ile Pro 230 235 240 245	774
tca act gtg aaa gct ggg gaa tta gaa aaa ata ata agt cgc tgt cag Ser Thr Val Lys Ala Gly Glu Leu Glu Lys Ile Ile Ser Arg Cys Gln 250 255 260	822
gtg tgc atg aag aaa aga cac tgagggccct attctatagt gtcacctaaa Val Cys Met Lys Lys Arg His 265	873
tgctagagct cgctgatcag cctcgac	900

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Ser Gly Ser Pro Ala Thr Trp Thr Thr Arg Gly Phe Val Phe Thr Arg 35 40 45
His Ser Gln Thr Thr Ala Ile Pro Ser Cys Pro Glu Gly Thr Val Pro 50 55 60

Leu Tyr Ser Gly Phe Ser Phe Leu Phe Val Gln Gly Asn Gln Arg Ala
 65 70 75 80
 His Gly Gln Asp Leu Gly Thr Leu Gly Ser Cys Leu Gln Arg Phe Thr
 85 90 95
 Thr Met Pro Phe Leu Phe Cys Asn Val Asn Asp Val Cys Asn Phe Ala
 100 105 110
 Ser Arg Asn Asp Tyr Ser Tyr Trp Leu Ser Thr Pro Ala Leu Met Pro
 115 120 125
 Met Asn Met Ala Pro Ile Thr Gly Arg Ala Leu Glu Pro Tyr Ile Ser
 130 135 140
 Arg Cys Thr Val Cys Glu Gly Pro Ala Ile Ala Ile Ala Val His Ser
 145 150 155 160
 Gln Thr Thr Asp Ile Pro Pro Cys Pro His Gly Trp Ile Ser Leu Trp
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 Lys Gly Phe Ser Phe Ile Met Phe Thr Ser Ala Gly Ser Glu Gly Ala
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INTERNATIONAL SEARCH REPORT

International Application No

PCT/US 00/08678

A. CLASSIFICATION OF SUBJECT MATTER

IPC 7 A61K38/39

According to international Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 7 A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

EPO-Internal, BIOSIS, PAJ, WPI Data, MEDLINE, CANCERLIT, CHEM ABS Data, EMBASE

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 5 856 184 A (SARRAS JR MICHAEL P ET AL) 5 January 1999 (1999-01-05) column 10, line 39 -column 11, line 12; claims 1-4; figures 7,8,10 column 12, line 14 - line 32 ---	1-4,9-16
X	PRESTAYKO ET AL: "Type IV collagen domains inhibit adhesion and migration of tumor cells and block angiogenesis", PROCEEDINGS OF THE ANNUAL MEETING OF THE AMERICAN ASSOCIATION FOR CANCER RESEARCH, US, PHILADELPHIA, PA: AACR, VOL. 39, PAGE(S) 45 XP002118641 abstract ---	1-4,9-16 -/-

Further documents are listed in the continuation of box C.

Patent family members are listed in annex.

° Special categories of cited documents :

- "A" document defining the general state of the art which is not considered to be of particular relevance
- "E" earlier document but published on or after the international filing date
- "L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
- "O" document referring to an oral disclosure, use, exhibition or other means
- "P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
2 August 2000	22/08/2000

Name and mailing address of the ISA
European Patent Office, P.B. 5818 Patentlaan 2
NL - 2280 HV Rijswijk
Tel. (+31-70) 340-2040, Tx. 31 651 epo nl,
Fax: (+31-70) 340-3016

Authorized officer

Noë, V

INTERNATIONAL SEARCH REPORT

International Application No

PCT/US 00/08678

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT		
Category	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 5 567 609 A (SARRAS JR MICHAEL P ET AL) 22 October 1996 (1996-10-22) abstract column 1, line 15 - line 24 column 4, line 15 - line 40 column 9, line 8 - line 19 ----	1-12
X	KEFALIDES N A ET AL: "SUPPRESSION OF TUMOR CELL GROWTH BY TYPE IV COLLAGEN AND A PEPTIDE FROM THE NC1 DOMAIN OF THE ALPHA3(IV) CHAIN" MEDICINA (BUENOS AIRES), vol. 59, no. 5-2, 1999, pages 553-553, XP002144122 the whole document ----	1,13
A	HAN JING ET AL: "A cell binding domain from the alpha-3 chain of type IV collagen inhibits proliferation of melanoma cells." JOURNAL OF BIOLOGICAL CHEMISTRY, vol. 272, no. 33, 1997, pages 20395-20401, XP002144123 ISSN: 0021-9258 page 20400, column 2, paragraph 2; table 2 ----	13-16
A	US 5 766 591 A (BROOKS PETER ET AL) 16 June 1998 (1998-06-16) abstract column 14, line 15 - line 23; examples 7,8,10 ----	1,9,13
A	SETTY SUMAN ET AL: "Interactions of type IV collagen and its domains with human mesangial cells." JOURNAL OF BIOLOGICAL CHEMISTRY, vol. 273, no. 20, 15 May 1998 (1998-05-15), pages 12244-12249, XP002144124 ISSN: 0021-9258 page 12245, column 1, paragraph 3 ----	3,8,12, 16
P,X	WO 99 49885 A (UNIV KANSAS MEDICAL CENTER) 7 October 1999 (1999-10-07) the whole document ----	1-16
P,X	PETITCLERC E ET AL: "NEW FUNCTION FOR NON-COLLAGENOUS DOMAINS OF HUMAN COLLAGEN" JOURNAL OF BIOLOGICAL CHEMISTRY, vol. 275, 2000, pages 8051-8061, XP002144125 abstract page 8052, column 1, paragraph 2 page 8055 -page 8057; figures 4-6,8 ----	1-4, 13-16

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

Continuation of Box I.2

Present claims 1-16 relate to an extremely large number of possible polypeptides. Support within the meaning of Article 6 PCT and/or disclosure within the meaning of Article 5 PCT is to be found, however, for only a very small proportion of the polypeptides claimed. In the present case, the claims so lack support, and the application so lacks disclosure, that a meaningful search over the whole of the claimed scope is impossible. Consequently, the search has been carried out for those parts of the claims which appear to be supported and disclosed, namely those parts relating to NC1 monomers of collagen type IV and their fragments.

The applicant's attention is drawn to the fact that claims, or parts of claims, relating to inventions in respect of which no international search report has been established need not be the subject of an international preliminary examination (Rule 66.1(e) PCT). The applicant is advised that the EPO policy when acting as an International Preliminary Examining Authority is normally not to carry out a preliminary examination on matter which has not been searched. This is the case irrespective of whether or not the claims are amended following receipt of the search report or during any Chapter II procedure.

INTERNATIONAL SEARCH REPORT

Information on patent family members			International Application No PCT/US 00/08678	
Patent document cited in search report	Publication date	Patent family member(s)		Publication date
US 5856184	A 05-01-1999	US 5691182 A US 5567609 A AU 3000895 A EP 0767676 A WO 9600582 A		25-11-1997 22-10-1996 25-01-1996 16-04-1997 11-01-1996
US 5567609	A 22-10-1996	AU 3000895 A EP 0767676 A WO 9600582 A US 5691182 A US 5856184 A		25-01-1996 16-04-1997 11-01-1996 25-11-1997 05-01-1999
US 5766591	A 16-06-1998	US 5753230 A AU 709645 B AU 1995295 A CA 2184493 A CN 1151120 A CZ 9602711 A EP 0754059 A FI 963692 A HU 76086 A JP 10500398 T NO 963894 A SK 119096 A WO 9525543 A ZA 9502214 A		19-05-1998 02-09-1999 09-10-1995 28-09-1995 04-06-1997 14-01-1998 22-01-1997 18-09-1996 30-06-1997 13-01-1998 18-11-1996 09-07-1997 28-09-1995 08-02-1996
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